

# **Synthesis of Novel Acceptor Substrates for Glycosyltransferase Enzymes.**

**Selena Guilly**



A thesis submitted for the degree of  
Doctor of Philosophy

University of Edinburgh

July 2004



*If you're down and confused  
And it's hard to do what you have to  
Concentration slips away  
'cos your baby is so far away  
Don't be angry, don't be sad  
Don't sit crying over good times you had  
'cos there's a rose in a fisted glove  
And the eagle flies with the dove*

*For Paul*



# Contents

*Aims*

*Abstract*

*Acknowledgements*

*Abbreviations*

<b>1. Introduction.</b>	<b>1</b>
<b>1.1. Glycoproteins.</b>	<b>1</b>
1.1.1. The structure and diversity of <i>O</i> - and <i>N</i> -linked oligosaccharides.	1
1.1.2. The role of oligosaccharides in modulating the biology of glycoforms.	4
1.1.3. Oligosaccharides and Lectins: their role in intercellular recognition and adhesion.	5
1.1.4. Function of sugar chains of glycoprotein hormones.	7
1.1.5. Oligosaccharides and the transport and folding of proteins.	9
<b>1.2. Biosynthesis of <i>N</i>-linked oligosaccharides.</b>	<b>11</b>
1.2.1. The lipid carrier for oligosaccharide assembly.	11
1.2.2. Construction of the lipid-linked heptasaccharide in the cytoplasm.	12
1.2.3. Completion of the lipid-linked tetradecasaccharide in the lumen.	15
1.2.4. <i>N</i> -linked protein glycosylation and processing.	17
<b>1.3. Chemical synthesis of oligosaccharides.</b>	<b>21</b>
1.3.1. Chemical synthesis of $\beta$ -1,2- <i>cis</i> -mannosyl linkages.	23
1.3.2. Epimerisation of $\beta$ -D-glucosides	24
1.3.3. Heterogeneous catalysis.	27
1.3.4. Intramolecular aglycon delivery.	29
1.3.5. Formation of $\beta$ -mannose linkages using glycosyl triflates and glycosyl phosphates.	30
<b>1.4. Chemoenzymatic synthesis of oligosaccharides.</b>	<b>33</b>
1.4.1. The Leloir pathway.	33
1.4.2. The use of enzymes from the Leloir pathway in chemoenzymatic synthesis.	34
1.4.3. The use of glycosidase enzymes in chemoenzymatic synthesis.	37
<b>1.5. The lipid carrier for oligosaccharide assembly: dolichyl phosphate.</b>	<b>40</b>
1.5.1. The occurrence, isolation and synthesis of dolichyl species.	40
<b>1.6. Conclusion.</b>	<b>43</b>

<b>2. Synthesis of Novel Lipid Analogues of Dolichyl Phosphate.</b>	<b>45</b>
2.1. Introduction to past research.	45
2.2. Synthesis of novel dolichyl analogues.	50
2.2.1. Synthesis of 3,7-dimethyl-8-(12-N-phthalimidododecanoyloxy)octyl phosphate.	50
2.2.2. Synthesis of 3,7-dimethyl-8-[12-(4'-dimethylaminonaphthalene-1'-sulfonamido)dodecanoyloxy]octyl phosphate.	58
2.3. Fluorescence spectra of the dansyl-amide lipid.	64
2.4. Biological testing of dolichyl lipid analogues.	70
2.5. Attempted synthesis of 3,7,11-trimethyl-12-(12-N-phthalimidododecanoyloxy)dodecyl phosphate.	72
2.6. Conclusion.	80
2.7. Future work.	81
<b>3. Synthesis of Lipid-Linked Carbohydrates and N-linked Chitbiose.</b>	<b>82</b>
3.1. Introduction.	82
3.2. Synthesis of phytanyl-linked chitobiose.	85
3.3. Synthesis of the phytanyl-linked trisaccharide, Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc.	88
3.4. Synthesis of phytanyl-linked glucosamine.	90
3.5. Enzyme-catalysed synthesis of phytanyl-linked chitobiose.	97
3.6. Conclusion.	99
3.7. Synthesis of orthogonally protected chitobiose.	100
3.8. Conclusion.	109
3.9. Future work.	109
<b>4. Experimental.</b>	<b>110</b>
4.1. General experimental.	110
4.1.1. Instrumentation.	110
4.1.2. Chromatography.	111
4.1.3. Solvents and Reagents.	111
4.2. Synthesis of lipid analogues of dolichyl phosphate.	112
4.3. Synthesis of lipid-linked monosaccharide and disaccharide.	137
4.4. Synthesis of protected chitobiose.	158
<b>5. Bibliography.</b>	<b>171</b>



## Aims.

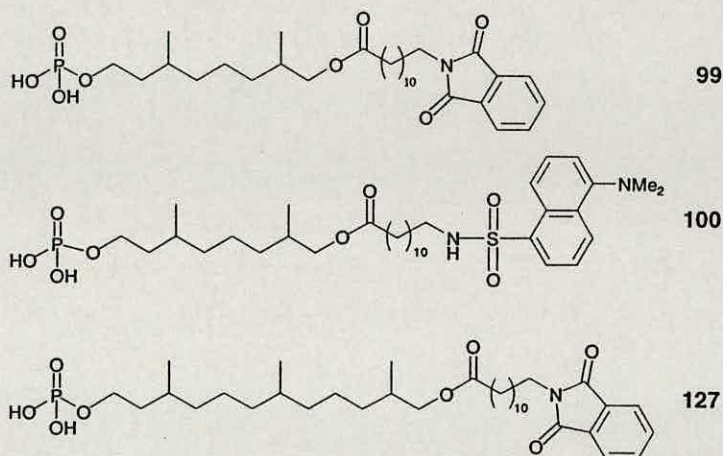
The aim of this project was to synthesise three novel analogues of the naturally occurring lipid, dolichyl phosphate (Dol-P). All of these lipids were designed to be multi-functionalised. An ester substituent was incorporated into the backbone of the lipid chain in order to increase their solubilities in water. Two of the lipids had an *N*-phthalamido group at the  $\omega$ -end of the chain, while the third bore a fluorescent probe at this point. The requirements for chain length and polyisoprenoid structure were then to be tested. It was reasoned that the fluorescent label would be of some aid in characterising the interactions of Dol-P or its glycosylated derivatives with enzymes which utilise these substrates or proteins participating in sugar transport across the cell membrane.

A phytanyl-linked monosaccharide was to be prepared to provide a cheaper alternative route to phytanyl pyrophosphoryl chitobiose. This compound was to be subsequently used to investigate the unknown mannosyltransferases active in the early stages of *N*-glycan biosynthesis.

Finally, fluorescently-labelled asparagine-linked chitobiose allowed research into the availability of an enzyme that could be used in the synthesis of an asparagine-linked trisaccharide.

## Abstract.

Our research has been based on the biosynthetic pathway to *N*-linked glycoproteins; species which are comprised of an oligosaccharide moiety attached to the side chain nitrogen atom of an asparagine residue in a peptide. In order to effectively study the steps involved in the biosynthesis of lipid-linked oligosaccharides and their subsequent transfer to a nascent protein in co-translational modification processes, it was essential to have a source of pure dolichols in sufficient quantities to allow the synthesis of both biosynthetic intermediates and synthetic analogues in the pathways responsible for the biogenesis of glycoproteins. Since supplies of naturally occurring dolichols are scarce and as they are difficult to synthesise, a number of lipid substrates were prepared instead as synthetic analogues of the naturally occurring dolichyl phosphate.



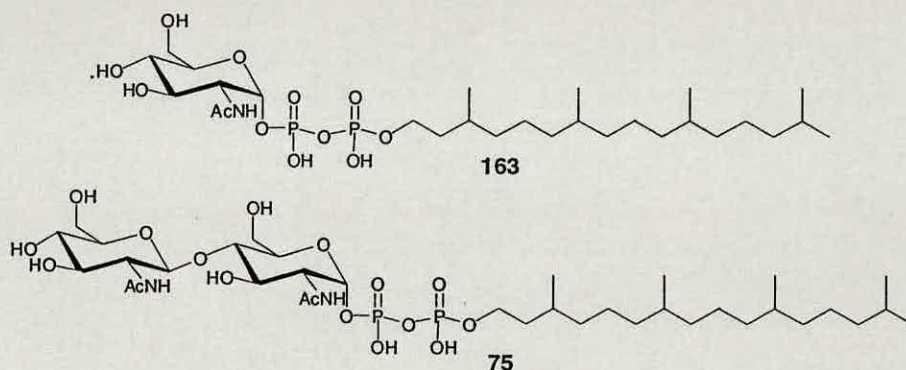
Two novel analogues of the naturally occurring lipid, dolichyl phosphate, were synthesised. The first of these, lipid **99**, was synthesised in seven steps from commercially available citronellol. The key step in this synthetic route involved creating an ester substituent in the backbone of the lipid chain to generate 8-benzyloxy-2,6-dimethyloct-2-en-1-yl 12-bromododecanoate, **106**, in 87% yield. Lipid **99** was found to exhibit 77% activity relative to phytanyl phosphate, **98**, in the biological assay with Dol-P-Man synthase.



A new synthetic route had to be devised in order to attach a fluorescent probe to the  $\omega$ -end of lipid **99** so that the dansyl amide lipid, **100**, could be prepared. Lipid **100** was also prepared from commercially available citronellol in an eight step route. The key transformations here were the introduction of an azide moiety at the  $\omega$ -end of the chain to afford 8-hydroxy-2,6-dimethyloctyl 12-azidododecanoate, **121**, in a yield of 89% and the reduction of the azide by a Staudinger reaction, followed by attachment of the dansyl sulfonamide to generate the fluorescent lipid di-tert-butyl 3,7-dimethyl-8-[12-(4'-dimethylaminonaphthalene-1'-sulfonamido)dodecanoxy]octyl phosphate, **123**, in 44% yield. Although lipid **100** showed only 14% activity relative to phytanyl phosphate in the biological assay with Dol-P-Man synthase, it was nevertheless clearly recognised and mannosylated by the enzyme. In addition, a fluorescence study of lipid **100** showed that this substrate is indeed strongly fluorescent. The environmentally sensitive fluorescent quantum yields and emission spectra that a dansyl amide provides will be useful for further study of the unknown mannosyltransferases active in the early pathway of *N*-glycan biosynthesis.

Finally, work towards the synthesis of an analogue of lipid **99**, namely lipid **127**, which incorporates an extra isoprene unit, was conducted.

Many of the enzymes involved in the biosynthesis of *N*-linked glycoproteins have already been isolated and characterised. However, some of those enzymes involved in the early stages of *N*-glycan biosynthesis remain unidentified. In order to gain more information about the unknown mannosyltransferases that are active in the early stages of *N*-glycan biosynthesis, a phytanyl-linked monosaccharide (glucosamine), **163**, and disaccharide (chitobiose), **75**, were prepared.



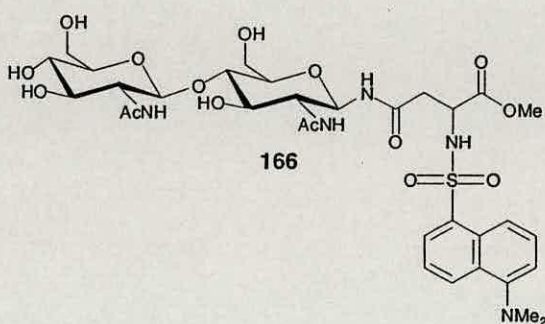
The synthesis of phytanyl-linked chitobiose, **75**, from commercially available chitobiose octaacetate, **70**, was successfully achieved in five steps. The most important step in this process was the synthesis of the  $\alpha$ -chitobiosyl phosphate dibenzyl ester, **72**, in 66% yield. Once this compound had been made it was coupled to phytanyl phosphate to generate the acetyl protected phytanyl pyrophosphoryl chitobioside, **74**, in 73% yield. Enzymatic transformation of this substrate was performed to generate a phytanyl-linked trisaccharide, **76**, which will be useful for providing biosynthetic intermediates for the studies of subsequent glycosyltransferases of the biosynthetic pathway of *N*-linked oligosaccharides.

A new method for preparing phytanyl-linked glucosamine, **163**, in an overall yield of 34% has also been developed. The yield obtained for each step in this synthetic route was excellent, with the exception of the anomeric deacetylation of peracetylated glucosamine. The low yield of this reaction is counteracted by the ease with which large quantities of 1-*O*-deacetylated glucosamine can be prepared. Although the attempted enzyme-catalysed transformation of phytanyl-linked glucosamine, **163**, to phytanyl-



linked chitobiose failed, it shows that the phytanyl-linked monosaccharide is not a suitable acceptor substrate for the first glycosyltransferase (*N*-acetylglucosaminyltransferase) in the biosynthetic pathway to *N*-linked glycoproteins.

Finally, work towards the preparation of a fluorescently-labelled asparagine-linked chitobiosyl substrate, **166**, is reported. Commercially available glucosamine hydrochloride, **176**, was converted into *O*-(2-phthalimido-2-deoxy-3,4-*O*-acetyl-6-benzyl- $\beta$ -D-glucopyranosyl)-(1,4)-2-phthalimido-2-deoxy-3-*O*-acetyl-6-benzyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **184**, in eight steps and an overall 16% yield. This work has formed the basis for the synthesis of a fluorescently-labelled asparagine-linked chitobiose substrate, **166**, that will be used to find a  $\beta$ -mannosyltransferase capable of directly mannosylating *N*-linked chitobiose.



## Acknowledgements.

I would like to thank Professor Sabine Flitsch for her supervision over the course of my research. Thanks are also due to the School of Chemistry, University of Edinburgh for funding this work. I would like to thank Dr. Alison Daines for taking the time to proof-read my thesis and for providing invaluable advice and help. Dr. Laurence Carmès and Dr. Derek MacMillan both deserve a special mention for the guidance and encouragement that they provided in the lab. Thanks too to Dr. Ines Sprung for undertaking the biological work that this project entailed.

This thesis would not be complete without thanking the excellent technical staff working in the School of Chemistry: John Millar and Wesley Kerr for the NMR services and Alan Taylor for the mass spectrometry. Thanks to Derek Burgess, Raymond Borwick, Tim Calder and Kenny Lorimer for running a very efficient and friendly stores service. I would like to thank all in lab 34/25 for making the time I spent there most enjoyable (even when we were freezing!), especially Denise, Ian, Alison, Kevin and Geoff.

I would like to thank my Mum and Dad for their continued support throughout the entire course of my studies and without whose advice and love I would not have gone this far. Finally, last but not least, I would like to thank Paul for always being there and for encouraging me when things were just too much!



## Abbreviations.

There follows a list of common abbreviations used in the text of this thesis.

AIBN	2,2'-Azobisisobutyronitrile
ALG	Asparagine-linked glycosylation
Asn	Asparagine
br	Broad
BSTFA	<i>N,O</i> -Bis(trimethylsilyl)trifluoroacetamide
<sup>n</sup> BuLi	<i>n</i> -Butyl lithium
CAN	Ceric ammonium nitrate
CDCl <sub>3</sub>	Deuteriochloroform
CDI	<i>N,N'</i> -Carbonyldiimidazole
d	Doublet
DAST	Diethylaminosulfur trifluoride
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
dd	Doublet of doublets
DDP	Dibenzyl <i>N,N'</i> -diethylphosphoramidite
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DME	1,2-Dimethoxyethane
DMF	Dimethylformamide
DNaseI	Deoxyribonuclease I
Dol-P	Dolichyl phosphate
DPM1	Dolichyl phosphate mannose synthase
E. Coli	Escherichia Coli
e.e.	Enantiomeric excess
ER	Endoplasmic reticulum

FAB	Fast atom bombardment
FSH	Follicle-stimulating hormone
Fuc	Fucose
Gal	Galactose
GalNAc	<i>N</i> -Acetylgalactosamine
GDP	Guanosine 5'-diphosphate
Glc	Glucose
GlcNAc	<i>N</i> -Acetylglucosamine
GPT	<i>N</i> -Acetylglucosamine phosphate transferase
h	Hour
hCG	human chorionic gonadotropin
His	Histidine
HRMS	High resolution mass spectrometry
Hz	Hertz
<i>J</i>	Spin coupling constant
kg	Kilogram
K <sub>m</sub>	Michaelis constant
LDA	Lithium diisopropylamide
LH	Luteinizing hormone
LRMS	Low resolution mass spectrometry
m	Multiplet
MAG	Myelin-associated glycoprotein
Man	Mannose
mCPBA	<i>meta</i> -Chloroperbenzoic acid
min	Minute
M <sub>p</sub>	Melting point
NBS	<i>N</i> -Bromosuccinimide
NDP	Nucleoside diphosphate
NeuAc	<i>N</i> -Acetylneuraminic acid
NIS	<i>N</i> -Iodosuccinimide

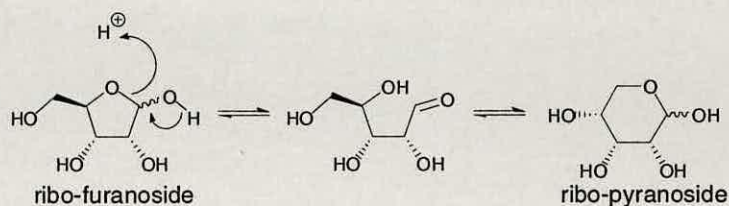
nm	Nanometre
NMR	Nuclear magnetic resonance
NTP	Nucleoside triphosphate
OT	Oligosaccharyl transferase
<i>p</i>	<i>Para</i>
PP	Pyrophosphate
ppm	Parts per million
q	Quartet
RER	Rough endoplasmic reticulum
R <sub>f</sub>	Retention factor
s	Singlet
Ser	Serine
t	Triplet
TBABr	Tetra- <i>N</i> -butylammonium bromide
TBPP	Tetrabenzyl pyrophosphate
td	Triplet of doublets
TES	Triethylsilane
TFA	Trifluoroacetic acid
Tf <sub>2</sub> O	Trifluoromethane sulfonic anhydride
Tg	Thyroglobulin
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin layer chromatography
tm	Triplet of multiplets
TrisHCl	Tris(hydroxymethyl)aminomethane
TSH	Thyroid-stimulating hormone
UDP	Uridine 5'-diphosphate glucose
UMP	Uridine 5'-monophosphate glucose
uv	Ultraviolet



# 1. Introduction.

## 1.1. Glycoproteins.

Glycoproteins are complexes in which carbohydrate structures are covalently attached to the backbone of a peptide chain. Although only seven or eight monosaccharides are commonly found as building blocks in mammalian systems, the multifunctionality of these monomers enables an immense variety of complex structures to be assembled.<sup>1</sup> Each of these commonly occurring monosaccharides is polyvalent and thus has the potential to be extended by glycosidic linkages to as many as five other sugar residues giving rise to the characteristic branched structures. In addition, each of the constituent monosaccharides may exist in one of two different anomeric conformations ( $\alpha$ - or  $\beta$ -), and may adopt either pyranose or furanose forms as depicted in Fig 1.1.



**Fig 1.1 :** *Ribose has the potential to exist as a five-membered oxygen heterocycle (furanose form), in an open-chain form or as a six-membered oxygen heterocycle (pyranose form).*<sup>2</sup>

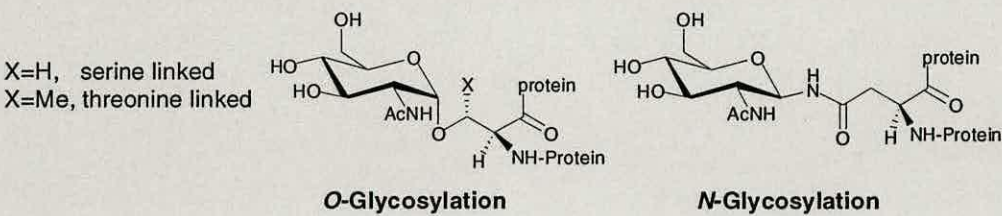
Consequently, the potential for structural diversity in oligosaccharides is immense, making them ideal for coding vast amounts of information in various biological recognition processes.

### 1.1.1. The structure and diversity of *O*- and *N*-linked oligosaccharides.

Despite the huge variations in the oligosaccharide side chains of glycoproteins, these compounds can be classified into two main categories: *O*- or *N*-glycans. Thus, the carbohydrates of glycoproteins are most commonly attached to either the side chain

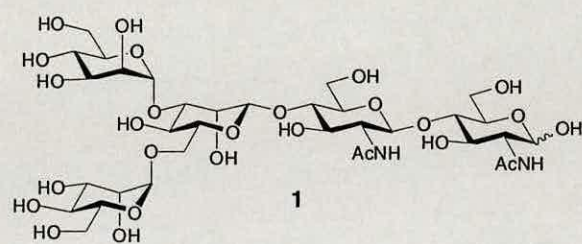


oxygen atom of serine or threonine residues by *O*-glycosidic linkages (*O*-glycan) or to the side chain nitrogen of asparagine by *N*-glycosidic linkages (*N*-glycan), Fig 1.2.



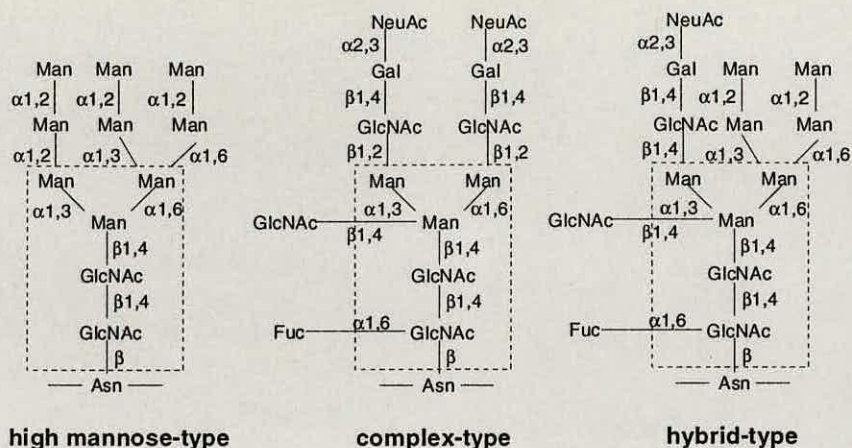
**Fig 1.2 :** *The two major types of glycosidic linkage found in eukaryotic glycoproteins.*

*N*-glycans are generally constructed around a common pentasaccharide core which consists of three mannose and two *N*-acetylglucosamine residues, 1, Fig 1.3.



**Fig 1.3 :** *The structure of the pentasaccharide core of N-glycans.*

*N*-linked oligosaccharides can be further classified as high-mannose-, complex-, hybrid-, or poly-*N*-acetylglucosamine-type depending upon the composition of the outer sugar chains, Fig 1.4.<sup>3</sup>



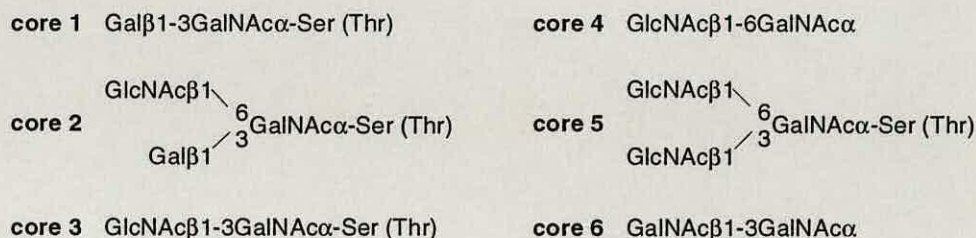
**Fig 1.4 :** Three subgroups of *N*-glycans: High mannose-, complex- and hybrid-type. The structure within the dashed line represents the pentasaccharide core found in all *N*-glycan species.

High mannose-type glycans contain only  $\alpha$ -mannosyl residues attached to this trimannosyl core. Complex-type glycans contain no mannose residues other than those in the trimannosyl core, but have branches with *N*-acetylglucosamine residues at their reducing termini attached to the core.<sup>4</sup> The number of branches usually ranges from two in a bi-antennary structure to four in a tetra-antennary structure. While various monosaccharides can be found in the branches, the presence or absence of fucose and a bisecting GlcNAc on the core contributes yet further to the enormous structural variation of complex-type glycans.<sup>4</sup>

The hybrid-type *N*-glycans have the characteristic features of both complex-type and high mannose-type glycans.<sup>4</sup> Here, one or two  $\alpha$ -mannosyl residues are linked to the Man $\alpha$ 1-6 arm of the trimannosyl core and usually one or two branches are linked to the Man $\alpha$ 1-3 arm of the core.<sup>4</sup> The fourth group is the poly-*N*-acetylglucosamine *N*-glycans containing repeating units of (Gal $\beta$ 1-4GlcNAc $\beta$ 1-3-) attached to the core. These repeats may also be branched. Poly-*N*-acetylglucosamine extensions are most frequently found in tetra-antennary glycans.<sup>1</sup> First discovered on erythrocyte membrane glycoproteins, these polylactosamine oligosaccharides may be substituted in various ways and have been shown to carry ABH and I<sub>I</sub> blood group antigens.<sup>5</sup>



In contrast to *N*-linked glycans, *O*-linked glycans do not share a common core structure. They are based around a number of different core structures which can be categorised into at least six groups, as illustrated in Fig 1.5.<sup>4</sup>



**Fig 1.5 :** Six types of core structures found in *O*-linked glycans.<sup>4</sup>

Although these glycans are often linked to serine or threonine residues through GalNAc, the linkages may also be through other residues such as fucose.<sup>4</sup> Since our research has been based around *N*-linked glycans, further discussion of *O*-glycans will not take place.

### 1.1.2. The role of oligosaccharides in modulating the biology of glycoforms.

The *O*- and *N*-glycans may contain different core structures but in each case it is the outer chain oligosaccharides attached to these cores that are known to control the biodistribution and biological properties of the parent protein or peptide.<sup>1</sup> Oligosaccharides can modify the intrinsic properties of the proteins to which they are attached by altering their stability,<sup>6</sup> protease resistance or quaternary structure.<sup>4</sup> The large size of oligosaccharides may allow them to cover functionally important areas of proteins, to modulate the interactions of glycoconjugates with other molecules, and to affect the rate of processes which involve conformational changes.<sup>4</sup> In the next few sections some examples of the functions of oligosaccharides will be discussed in order to highlight their importance as biological molecules.

### **1.1.3. Oligosaccharides and lectins: Their role in intercellular recognition and adhesion.**

Essential roles for oligosaccharides have been identified in a number of intercellular recognition and adhesion events. Lectins are carbohydrate specific proteins that mediate cellular recognition.<sup>7</sup> Each lectin molecule contains two or more carbohydrate-combining sites. When they react with cells, for example erythrocytes, they will not only combine with the sugars on their surfaces, but also cause crosslinking of the cells and their subsequent precipitation; a process known as cell agglutination. This erythrocyte agglutinating, or haemagglutinating, activity of lectins is used routinely for their detection and characterisation.<sup>8</sup>

Lectins also act as recognition determinants in other diverse biological processes. These include clearance of glycoproteins from the circulatory system, control of intracellular traffic of glycoproteins, adhesion of infectious agents to host cells, recruitment of leukocytes to inflammatory sites and intercellular interactions in the immune system in malignancy and metastasis.<sup>8</sup>

Most lectins can be classified into one of three categories: (1) Simple, (2) mosaic (or multi-domain) and (3) macromolecular assemblies, although borderline cases exist.<sup>8</sup> Within each category, lectins can be grouped into distinct families with similar sequences and structural properties.<sup>8</sup>

Simple lectins consist of a small number of sub-units, not necessarily identical, which may contain an additional domain besides their carbohydrate binding site(s). This class comprises practically all known plant lectins as well as the galectins, a family of galactose-specific animal lectins.<sup>8</sup>

Included in the mosaic (or multi-domain) group are a number of diverse proteins from different sources, ranging from viral haemagglutinins to C-, P- and I-type animal lectins.<sup>8</sup> They are all composite molecules with a wide range of molecular



weights, consisting of several kinds of protein modules or domains, only one of which possesses a carbohydrate binding site.<sup>8</sup>

Viral and microbial surface lectins mediate the adhesion of the organisms to host's cells, a pre-requisite for infection to occur.<sup>9</sup> This was first demonstrated in the 1940's for the influenza virus haemagglutinin. This lectin is specific for *N*-acetylneuraminic acid, a sialic acid. Removal of this sugar from the cell membranes by sialidase abolishes viral binding and prevents infection, while enzymatic re-attachment of *N*-acetylneuraminic acid or insertion of sialic acid-containing oligosaccharides (for example in the form of glycolipids) into the membranes of sialidase-treated cells restores the ability of the cells to bind the virus and be infected.<sup>7</sup>

I-type lectins are characterised by variable numbers of extracellular immunoglobulin-like domains.<sup>8</sup> The most important and well-characterised lectins of this type are the sialoadhesins, a family of sialic acid specific type I membrane glycoproteins.<sup>10</sup> They include the macrophage receptor that mediates the adhesion of these cells to erythrocytes (sialoadhesin), the lymphocyte surface antigen CD22 found only on B cells, CD33 present on early myeloid cells, and a myelin-associated glycoprotein, MAG.<sup>8</sup> I-type lectins have been implicated in cell-cell interactions: Sialoadhesin and CD22 in those of the immune system and MAG in the maintenance of myelin and in neuronal regeneration.<sup>8, 11</sup>

C-type lectins require  $\text{Ca}^{2+}$  for activity.<sup>12</sup> This class of lectins includes over fifty members in three different families, namely endocytic lectins, collectins and selectins. The selectins mediate the adhesion of circulating leukocytes to endothelial cells of blood vessels, a pre-requisite for the exit of leukocytes from the circulatory system and their migration into tissues.<sup>13</sup> They, thus, control leukocyte trafficking to sites of inflammation and the migration of lymphocytes to specific lymphoid organs.<sup>13</sup> There are three types of selectins: E-selectin, P-selectin and L-selectin, all highly asymmetric membrane-bound proteins.<sup>8</sup> L-selectin is found on all leukocytes and is involved in the re-circulation of lymphocytes, directing them specifically to peripheral lymph nodes. E- and P-selectins are expressed on endothelial cells only



when these cells are activated by inflammatory mediators released from tissue cells in response to infection. In cases of microbial infection, the selectin-mediated adhesion of leukocytes to activated endothelial cells is a major factor in clearance of the infectious agents.<sup>14</sup> It therefore plays an important role in immune defence by recognising and targeting carbohydrates on invading pathogens.<sup>14</sup>

Macromolecular assembly-type lectins are common in bacteria in the form of fimbriae (or pili).<sup>8</sup> These are heteropolymeric organelles present on the surface of the bacteria, consisting of helically arranged sub-units of several different types.<sup>8</sup> Only one of the sub-units possesses a carbohydrate binding site. The lectin-carbohydrate interactions in bacterial infections were investigated by experiments which showed that blocking lectins using suitable sugars provided protection against bacterial infection.<sup>14</sup> It was found that mannose and methyl- $\alpha$ -mannoside specifically inhibited infection of the urinary tract of mice and rats by different strains of type 1 fimbriated *E. Coli* and *Klebsiella pneumoniae* respectively, while *N*-glycosylneuraminic acid containing glycopeptides, administered orally, protected colostrum-deprived newborn calves against lethal doses of enterotoxigenic *E. Coli* K99.<sup>14</sup> Similarly, *N*-acetylneuraminic acid considerably reduced colonisation of the lung, liver and kidney by *Pseudomonas aeruginosa* administered intravenously to the animal.<sup>14</sup> These findings illustrate the great potential of carbohydrates in the prevention of infections caused by bacteria that express surface lectins and provide a basis for the development of anti-adhesion therapy of microbial infections.

#### **1.1.4. Function of sugar chains of glycoprotein hormones.**

Four glycoprotein hormones have been found in a variety of mammals. Three of these are produced in the anterior pituitary: Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are produced by the gonadotrophs, while thyroid-stimulating hormone (TSH) is synthesised by thyrotrophs.<sup>15, 16</sup> LH and FSH have general biological roles in the stimulation of testicular and ovarian functions, while TSH regulates the synthesis and secretion of thyroid hormones.<sup>17</sup> The fourth glycohormone, human chorionic gonadotropin (hCG), is produced by trophoblasts in



the placenta. These hormones, with their diverse physiological functions, are closely related in structure with each consisting of two peptide chains designated  $\alpha$  and  $\beta$ .<sup>17</sup> Both sub-units are glycosylated at specific residues and are highly cross-linked internally by disulphide bonds.<sup>17</sup>

Much research has been conducted to investigate the role of the sugar portion of glycoprotein hormones.<sup>17</sup> The effect that deglycosylation of hCG had on the activity of glycohormones was studied by Kalyan and Bahl.<sup>18</sup> It was found that partial removal or modification of the carbohydrate portion of hCG resulted in impairment of the biological activity without affecting its binding to the receptor.<sup>18</sup> Complete removal of carbohydrate from hCG, without any detectable adverse effect on the polypeptide chains, was achieved by treating the sub-units with trifluoromethane sulfonic acid. The deglycosylated hCG failed to stimulate hormonal activity and, in addition, the deglycosylated material was found to compete with native hCG for receptor sites and act as a potent inhibitor of biological activity.<sup>18</sup>

Further study of the functional role of the sugar moiety of hCG was conducted. The adenyl cyclase of rat corpora luteal membrane was increased in a linear fashion in response to the addition of hCG.<sup>19</sup> Addition of glycopeptides, obtained from hCG by exhaustive pronase digestion, to this reaction mixture inhibited the adenyl cyclase activation by hCG in a dose-dependent manner. This indicated that a membrane lectin, which binds to the sugar chains of hCG, may be involved in the regulation of luteal cell hCG-stimulated adenyl cyclase.<sup>19</sup>

For the remaining glycohormones, LH, FSH and TSH, studies have shown that deglycosylation was associated with increased binding to the receptor sites and that the deglycosylated hormones were potent competitive inhibitors of binding of the native hormone.<sup>20</sup> These data suggest that the *N*-linked oligosaccharides of glycohormones play a crucial role in regulating the biological activity of hormones by participating in signal transduction once the hormone has been bound to the receptor.<sup>20</sup>



### 1.1.5. Oligosaccharides and the transport and folding of proteins.

The carbohydrate side chains of glycoproteins are known to influence the early stages of polypeptide folding, to ensure correct secretion and to maintain subsequent solubility and conformation of the polypeptide.<sup>21</sup> The endoplasmic reticulum (ER) is the site of synthesis of membrane and secretory proteins. As they enter the lumen of the ER during the translocation process, newly synthesised membrane and secretory proteins are subjected to the action of enzymes and molecular chaperones that assist their conformational maturation.<sup>22</sup>

Among the molecular chaperones of the ER are two related lectin-like chaperones, calnexin and calreticulin. Calnexin is a type I membrane protein which has been demonstrated to bind transiently to several glycoproteins via partially trimmed *N*-linked oligosaccharide chains which carry a single glucose residue.<sup>22, 23</sup> The association of calnexin with its substrate glycoproteins facilitates their folding and oligomerisation, and supports quality control.<sup>24</sup>

Calreticulin is a soluble, luminal protein of the ER that possesses chaperone and quality control functions similar to those of calnexin.<sup>25</sup> It is a highly conserved ubiquitous protein and has been implicated in  $\text{Ca}^{2+}$  storage and intracellular signalling in the ER.<sup>25</sup> The chaperone function of calreticulin is assisted by two enzymes with contrasting catalytic activities: UDP-glucose: glycoprotein glucosyltransferase and glucosidase.<sup>26</sup> The sequential action of glucosidase I and II removes two glucose residues from  $\text{Glc}_3\text{Man}_5\text{GlcNAc}_2$  of *N*-linked glycoproteins in the ER, whereas UDP-glucose: glycoprotein glucosyltransferase catalyses the addition of glucose to the non-reducing end of mannose of the  $\alpha 1\text{-}3$  arm of  $\text{Man}_5\text{GlcNAc}_2$  chains in unfolded glycoproteins, thereby acting as a folding sensor.<sup>26, 27</sup> Repeated cycles of re-glucosylation by the glucosyltransferase lead to prolonged association of calreticulin with the unfolded protein until its appropriate folding occurs.<sup>26</sup> Should this process fail, the protein is retrotranslocated to the cytosol and subsequently degraded by the proteasome.<sup>28</sup> Substrate studies have identified the single terminal glucose residue as a critical determinant recognised by calreticulin



because oligosaccharides containing 0, 2 or 3 glucose residues fail to bind.<sup>21</sup> Oligosaccharide binding is critical for the formation of complexes between glycoproteins and calreticulin.

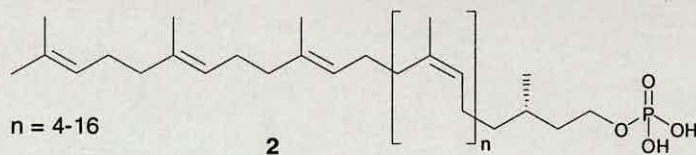
The occurrence of substrate-calnexin-calreticulin ternary complexes for an endogenous protein in the ER of live cells was investigated using newly synthesised thyroglobulin (Tg).<sup>22</sup> Results indicated that, in FRTL-5 cells (a continuous, cloned line of thyroid differentiated cells), calnexin and calreticulin interact with Tg in a carbohydrate-dependent manner, with largely overlapping kinetics that are concomitant with the mutation of Tg intrachain disulphide bonds, preceeding Tg dimerisation and exit from the ER.<sup>22</sup> Calreticulin was found to co-precipitate more with newly synthesised Tg than calnexin did. However, both were found in ternary complexes with Tg.<sup>22</sup> Treatment with thapsigargin (a specific inhibitor of ER  $\text{Ca}^{2+}$ -ATPases) induced the premature exit of Tg from the calnexin/calreticulin cycle, while stabilising and prolonging interactions of Tg with BiP (immunoglobulin heavy chain binding protein) and GRP94 (glucose-regulated protein 94), two chaperones whose binding is not carbohydrate-dependent.<sup>22</sup> This indicates that calnexin and calreticulin, acting in ternary complexes with a large glycoprotein substrate such as Tg, might be engaged in the folding of distinct domains while  $\text{Ca}^{2+}$  influences the folding of exportable glycoproteins, in part by regulating the balance of the substrate binding to different molecular chaperone systems within the ER.<sup>22</sup>

## 1.2. Biosynthesis of *N*-linked oligosaccharides.

The *N*-linked protein glycosylation pathway in eukaryotes can be divided into two different processes: (1) The assembly of the lipid-linked oligosaccharide at the membrane of the endoplasmic reticulum (ER); and (2) the transfer of the oligosaccharide from the lipid anchor, dolichyl pyrophosphate, to selected asparagine residues of nascent polypeptides.

### 1.2.1. The lipid carrier for oligosaccharide assembly.

Dolichyl phosphate (Dol-P), **2**, acts as the carrier in the assembly of pyrophosphate-linked oligosaccharides, and is also the acceptor in the synthesis of the sugar donors dolichyl phosphate mannose (Dol-P-Man) and dolichyl phosphate glucose (Dol-P-Glc), from guanosine 5'-diphosphate mannose (GDP-Man) and uridine 5'-diphosphate glucose (UDP-Glc) respectively.<sup>29</sup> The availability of dolichyl phosphate represents a key factor in the assembly of lipid-linked oligosaccharides, Fig 1.6.<sup>29</sup>



**Fig 1.6 :** *The lipid carrier for oligosaccharide assembly, dolichol phosphate.*

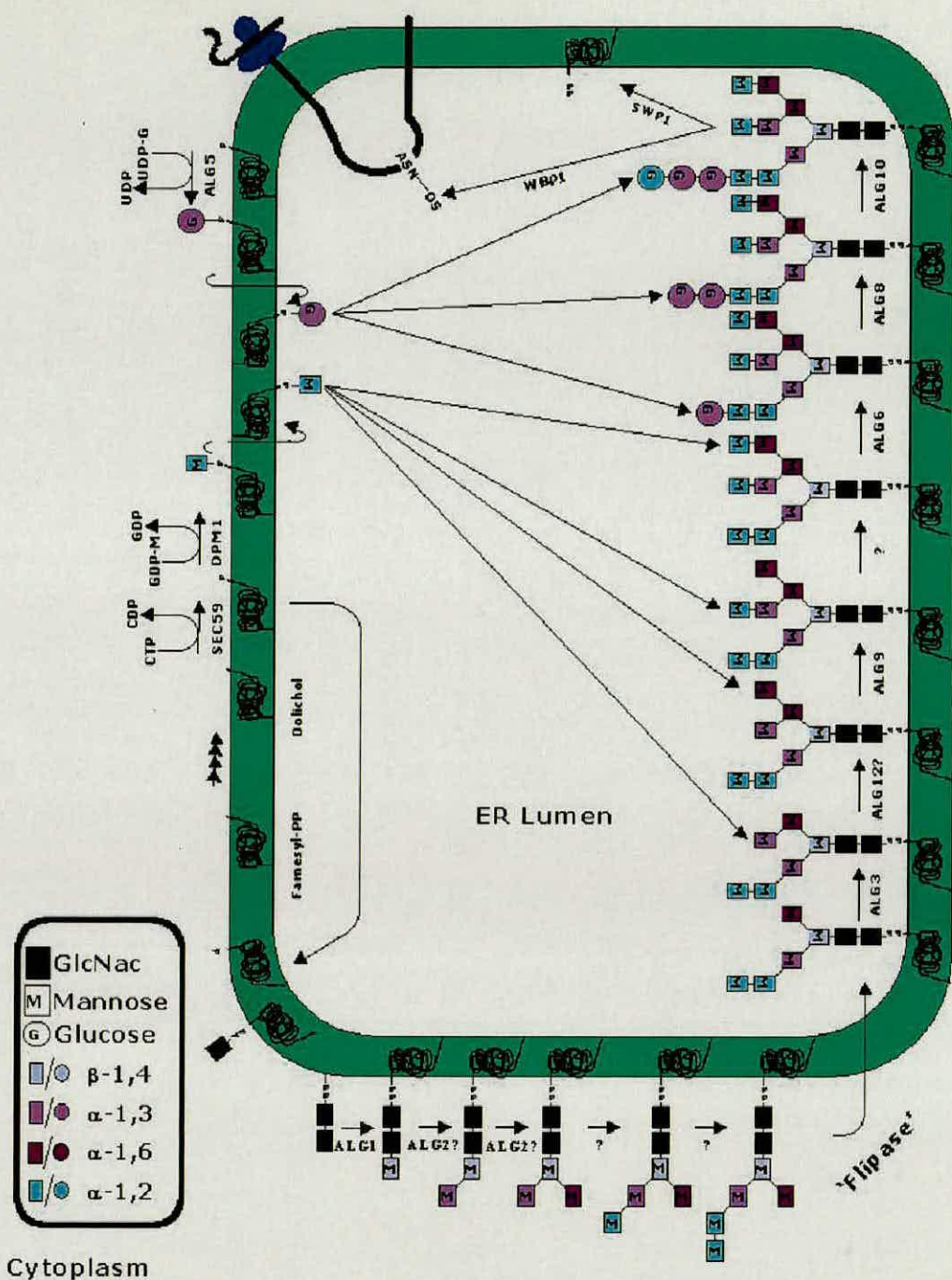
The dolichols are a family of naturally occurring polyisoprenoids of varying chain length. Dolichol is synthesised from farnesyl pyrophosphate by sequential additions of the activated isoprene unit, isopentenyl pyrophosphate. The length of dolichol molecules is species-dependent, but the mechanism determining the length is not known.<sup>29</sup> It has been proposed that chain elongation is terminated by the addition of isopentenol instead of isopentenyl pyrophosphate.<sup>30</sup> Alternatively, chain elongation may simply be terminated when the product contains a certain number of isoprene units and is therefore no longer a substrate for the *cis*-isoprenyltransferase.<sup>31</sup> Phosphorylation of dolichol is catalysed by the enzyme sec59p.



### 1.2.2. Construction of the lipid-linked heptasaccharide in the cytoplasm.

The biosynthesis of the lipid-linked oligosaccharide begins at the cytoplasmic side of the ER membrane with the addition of an *N*-acetylglucosamine phosphate (GlcNAc phosphate) residue to Dol-P and concomitant release of uridine 5'-monophosphate (UMP) as shown in Fig 1.7. This key process is mediated by *N*-acetylglucosamine phosphate transferase (GPT), an enzyme encoded by the ALG7 locus. The GlcNAc phosphate, which is added to the Dol-P moiety, is derived from the activated sugar nucleoside phosphate UDP-GlcNAc.<sup>29</sup> Studies have shown that the synthesis of GlcNAc-PP-Dol is a vital step in the synthesis of glycoproteins containing *N*-linked oligosaccharides.<sup>32</sup> When this reaction was inhibited by incubation of cells with the antibiotic tunicamycin, glycoproteins containing no *N*-linked oligosaccharides were formed.<sup>32</sup>

The next stage in the biosynthesis of *N*-linked glycoproteins is the addition of a second  $\beta$ -1,4-*N*-acetylglucosamine residue to produce the chitobiosyl-pyrophosphoryl-dolichyl unit, GlcNAc<sub>2</sub>-PP-Dol. This step has not been characterised in yeast nor in higher eukaryotic cells.<sup>29</sup> Expression studies in *E. Coli* have shown that ALG1 encodes a  $\beta$ -1,4-mannosyltransferase, which catalyses the next step in the synthesis, the transfer of the first mannose residue from GDP-Man to GlcNAc<sub>2</sub>-PP-Dol.<sup>33</sup> This enzyme is unique, introducing the only  $\beta$ -mannose residue known to exist in mammalian oligosaccharides. The preference for  $\beta$ -linked mannose at this point in the common structure of *N*-glycans may have arisen because the  $\beta$ -glycoside contributes significantly to the structural rigidity of the pentasaccharide core.<sup>34</sup> It has also been shown that the ALG1 protein accepts chitobiose attached to a phytanyl rather than a dolichyl carrier as a substrate, suggesting that dolichol is of limited importance for substrate recognition by this enzyme.<sup>35</sup>

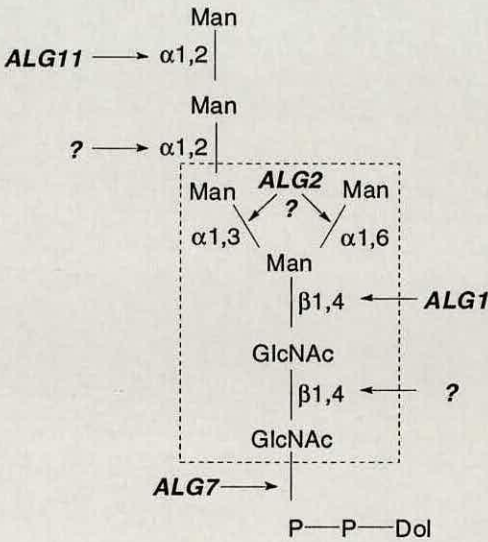


**Fig 1.7 : Biosynthesis of N-linked glycoproteins.**

The next few steps in the biosynthesis of N-linked glycoproteins that occur on the cytoplasmic side of the ER involve the attachment of four mannose residues to the lipid-linked trisaccharide, Man-GlcNAc<sub>2</sub>-PP-Dol, leading to the synthesis of a lipid-linked heptasaccharide, Man<sub>5</sub>GlcNAc<sub>2</sub>-PP-Dol. Studies have shown that *alg2* mutant



cells accumulate  $\text{Man}_2\text{GlcNAc}_2\text{-PP-Dol}$ , suggesting that the ALG2 protein is a mannosyltransferase active in the early pathway of oligosaccharide assembly.<sup>36, 37</sup> Studies have also resulted in the characterisation of ALG11, a yeast glycosylation mutant that is defective in the last step of the synthesis of the  $\text{Man}_5\text{GlcNAc}_2\text{-PP-Dol}$  core oligosaccharide on the cytosolic face of the ER.<sup>37</sup> Analysis of lipid-linked oligosaccharides and those on ALG11 glycoproteins define ALG11p to be involved in adding the final  $\alpha$ -1,2-linked Man to  $\text{Man}_4\text{GlcNAc}_2\text{-PP-Dol}$ .<sup>37</sup> Additional mannosyltransferases required for the assembly of  $\text{Man}_5\text{GlcNAc}_2\text{-PP-Dol}$  remain to be identified. Within this heptasaccharide can be seen the pentasaccharide core common to all *N*-linked oligosaccharides, Fig 1.8.



**Fig 1.8 :** The dolichyl pyrophosphate-linked heptasaccharide,  $\text{Man}_5\text{GlcNAc}_2\text{-PP-Dol}$ . The linkage of each individual glycosyl residue and the known loci coding for the corresponding glycosyltransferases are indicated. The structure within the dashed line represents the pentasaccharide core found in all *N*-glycan species.<sup>38</sup>

### 1.2.3. Completion of the lipid-linked tetradecasaccharide in the lumen.

Once the lipid-linked heptasaccharide has been synthesised on the cytoplasmic face of the ER, it is translocated across the lipid bilayer to the luminal side of the ER. The mechanism of transmembrane migration is not fully understood although protein-catalysed translocation has been postulated.<sup>29, 39</sup> In the lumen of the ER, a further seven sugar residues (four Man and three Glc) are added to the lipid-linked heptasaccharide. The addition of four mannose residues followed by three glucose residues is believed to occur in a stepwise fashion.<sup>40</sup> The late glycosyltransferases operating in the ER lumen differ from the glycosyltransferases that catalyse the reactions on the cytoplasmic side.<sup>29</sup> The former use dolichol-bound monosaccharides instead of nucleotide-activated monosaccharides as sugar donors and the genes coding for these transferases are non-essential for viability.<sup>29</sup>

The ALG3 gene encodes the enzyme that initiates the dolichyl-P-monosaccharide-dependent glycosylation reactions at the luminal side of the ER.<sup>41</sup> This enzyme is a Dol-P-Man dependent  $\alpha$ -1,3-mannosyltransferase and is involved in the addition of the first mannose residue to the heptasaccharide on the luminal side.<sup>41</sup> It is postulated that ALG9 encodes an  $\alpha$ -1,2-mannosyltransferase which adds a mannose residue to the  $\alpha$ -1,3-linked mannose.<sup>40</sup> The addition of the  $\alpha$ -1,6-linked mannose is probably catalysed by the ALG12 protein because *alg12* cells accumulate Man<sub>7</sub>GlcNAc<sub>2</sub>-PP-Dol which contains the  $\alpha$ -1,3- $\alpha$ -1,2 dimannose antenna.<sup>29</sup> The locus encoding the mannosyltransferase which adds the  $\alpha$ -1,2-linked mannose residue to the  $\alpha$ -1,6- arm has not yet been identified.<sup>29</sup> The resulting undecasaccharide is illustrated in Fig 1.9.



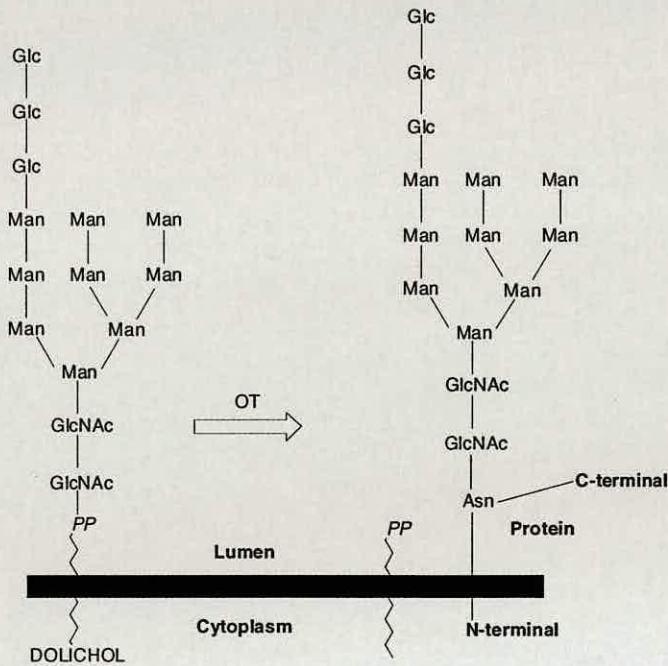


mannose residues has little effect on protein glycosylation.<sup>5</sup> In protozoa, the completed lipid-linked oligosaccharides contain nine mannoses,<sup>45</sup> seven mannoses<sup>46</sup> and six mannoses.<sup>47</sup>

Although an incompletely assembled lipid-linked oligosaccharide can be transferred to the protein, the efficiency of transfer is much less than if the lipid-linked oligosaccharide had been correctly constructed.<sup>29</sup> It has also been proposed that glucosylation protects the lipid-linked oligosaccharides from degradation.<sup>48</sup> A study of intact rat spleen lymphocytes which incorporate nucleotide sugars into lipid-linked oligosaccharides found that the non-glucosylated lipid-linked oligosaccharides were selectively degraded by a phosphodiesterase.<sup>48</sup>

#### 1.2.4. N-linked protein glycosylation and processing.

Once synthesised, the sugar chain is ready to be delivered from the lipid anchor to the protein yielding the asparagine-linked structure, Fig 1.10.



**Fig 1.10 :** *OT catalysed transfer of the dolichyl pyrophosphate-linked tetradecasaccharide to an asparagine side chain within a nascent polypeptide in the lumen of the RER.*<sup>49</sup>



*N*-linked glycosylation is catalysed by oligosaccharyl transferase (OT), a membrane associated, multimeric enzyme localised in the lumen of the ER.<sup>50</sup> In the reaction catalysed by OT, a complex oligosaccharide is transferred from a lipid-linked pyrophosphate donor to a nascent polypeptide chain as the peptide is translocated into the lumen of the ER.<sup>39</sup>

The peptide primary sequence requirements for glycosylation are minimal; the asparagine residue must be within the consensus sequence Asn-Xaa-Thr/Ser (NXT/S) where Xaa can be any of the 20 natural amino acids except proline.<sup>51</sup> The exclusion of NPT/S sequences suggests that local secondary structures play an important role in determining the outcome of glycosylation.<sup>51</sup> This theory is reinforced by the fact that 10-30% of all NXT/S sequences remain unglycosylated in mature proteins, even though they meet the primary sequence requirements.<sup>52</sup> Proteins that are destined for glycosylation are synthesised by membrane-associated ribosomes with an initial signal peptide, which guides the newly synthesised protein through the intracellular membrane bilayer into the lumen of the ER.<sup>50</sup> The signal peptide is subsequently cleaved by a signal peptidase.<sup>50</sup> The NXT/S sequence must clear the luminal side of the membrane bilayer by 12-14 residues before glycosylation can occur. However, the nascent polypeptide remains bound to the membrane of the ER and is likely to be only locally folded during OT mediated catalysis.<sup>50, 53</sup>

In addition to the asparagine residue, the absolute requirement for an unmodified hydroxyl amino acid implies a direct role for the hydroxyl group in catalysis.<sup>50</sup> Studies have found that formation of a hydrogen bond with the side chain of the hydroxyl amino acid was an absolute requirement in the glycosylation reaction mechanism.<sup>5, 54</sup> A model has been proposed in which a hydrogen-bond interaction between the amide of asparagine (the hydrogen-bond donor) and the oxygen of the hydroxyl group of the hydroxyl amino acid (the hydrogen acceptor) increased the nucleophilicity of the amide electron pair, resulting in a higher reactivity toward the glycosyl donor.<sup>5</sup> It was also observed that  $\beta$ -turns represent spatial arrangements of the peptide chain that favour the required hydrogen bonded contacts.<sup>5, 51</sup> Proline-



containing peptide analogues that did not serve as acceptors were unable to achieve a conformation that allowed the necessary hydrogen bonding.<sup>5</sup>

It would appear that the ability of the peptide to achieve a favourable conformation is necessary, not alone to provide the correct hydrogen bonding, but to provide adequate accessibility to the OT enzyme.<sup>5</sup> Since glycosylation occurs co-translationally, the asparagine that is to be glycosylated is part of a growing peptide chain that is in the process of folding.<sup>5</sup> Consequently, the period of time during which glycosylation can occur may be quite brief. Once the protein has folded, potential glycosylation sites are no longer accessible to the OT enzyme.<sup>55</sup>

Another factor in determining the level of glycosylation is the availability of the lipid-linked oligosaccharide donor.<sup>5</sup> Carson *et al.* found that added Dol-P increased the glycosylation of secreted RNase from 12% to 90% in bovine pancreas tissue slices.<sup>56</sup> Thus, Dol-P availability may regulate asparagine-linked glycosylation under some circumstances.

In summary, efficient glycosylation of proteins is dependent on a sufficient pool of completely assembled and glucosylated lipid-linked oligosaccharide donor, an adequate activity of OT, and a properly oriented and accessible Asn-Xaa-Thr/Ser sequence in the acceptor.<sup>5</sup>

Following the transfer to an asparagine residue within the protein, the core tetradecasaccharide, Glc<sub>3</sub>-Man<sub>9</sub>-GlcNAc<sub>2</sub>, is immediately processed in a series of reactions which invariably cleave the three terminal glucose and four mannose residues. Processing is initiated by the removal of the terminal glucose residue by  $\alpha$ -1,2-glucosidase I which is specific for this cleavage.<sup>5</sup> The two inner glucose residues are then removed by  $\alpha$ -1,3 specific glucosidase II.<sup>5</sup> Glucosidase I has been purified from calf liver while glucosidase II has been purified from rat liver and kidney.<sup>57, 58</sup> Evidence that glucosidase II removes both  $\alpha$ -1,3-glucose residues from Glc<sub>2</sub>Man<sub>9</sub>GlcNAc<sub>2</sub> has come from studies with the purified enzyme and from the finding that extracts of a glucosidase II-deficient mouse lymphoma cell line are



unable to remove glucose from either  $\text{Glc}_2\text{Man}_9\text{GlcNAc}_2$  or  $\text{Glc}_1\text{Man}_9\text{GlcNAc}_2$ .<sup>59</sup> These glucosidases are located in the membranes of the RER, as is a specific  $\alpha$ -mannosidase which catalyses the removal of at least one  $\alpha$ -1,2-linked mannose residue.<sup>5</sup>

The processed glycoprotein,  $\text{Man}_{5-8}\text{GlcNAc}_2\text{-Asn}$ , is subsequently translocated to the Golgi apparatus where glycosyltransferases catalyse the elaboration of the glycoprotein through transfer of additional monosaccharides such as fucose, galactose and sialic acid from the corresponding nucleotide diphosphates.<sup>50</sup> These reactions act in concert to produce the diverse *N*-linked glycoconjugate structures characteristic of mature proteins. It is the final oligosaccharide sequence that often determines the cellular destination of the assembled glycoprotein.<sup>50</sup>

It has been shown that some of the essential enzymes in *N*-linked protein glycosylation, for example *N*-acetylglucosamine phosphate transferase, are highly conserved between lower and higher eukaryotes.<sup>29, 60</sup> OT enzymes from yeast and higher eukaryotes also contain components with significant sequence similarity, suggesting a conservation of the enzyme.<sup>61</sup> Thus, it is believed that the process of *N*-glycosylation follows a highly conserved pathway.<sup>29</sup> This is also demonstrated in the fact that the asparagine-linked oligosaccharide structures have the common pentasaccharide core structure, as they all arise from the same biosynthetic precursor lipid-linked oligosaccharide.

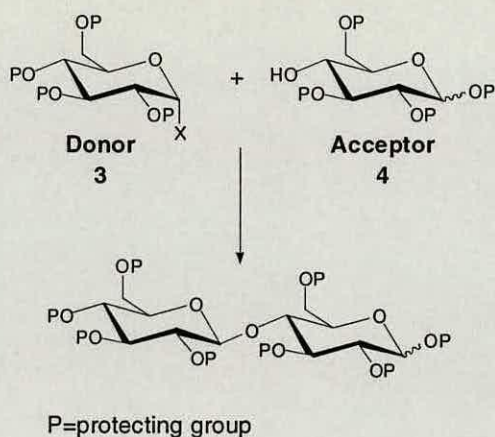
### 1.3. Chemical synthesis of oligosaccharides.

The abundance of carbohydrates in Nature and their diverse roles in biological systems make them attractive subjects for chemical and biological research. As has already been discussed, oligosaccharides play essential roles in a number of intercellular recognition and adhesion events. Carbohydrate residues can influence the stability, conformation, protease lability, thermal properties and solubility of proteins.<sup>62</sup> They have been implicated in regulation of peptide folding, viral replication, infection, immune response, and in general are known to control the biodistribution and biological properties of their carrier molecule.<sup>4</sup>

The synthesis of oligosaccharides, however, is not an easy task. The structural diversity that makes oligosaccharides such appealing target molecules brings with it problems of regio- and stereoselectivity unique to this field of chemistry. A comprehensive strategy must be decided upon prior to each novel synthesis, for oligosaccharide formation does not depend only on the size of the target structure but also on its linkages and the composition of its building blocks.

In the formation of a glycosidic linkage, two polyfunctional reaction partners (a donor and an acceptor) must be coupled.<sup>63</sup> Orthogonal protection of these substrates is necessary to allow selective blocking and unblocking of reactive sites and thus ensure regioselectivity of the glycosidation reaction. The reactivities at the anomeric centre of the glycosyl donor and the hydroxyl group in the glycosyl acceptor depend heavily on the nature of the protecting groups in each component sugar. Conformational and steric factors, as well as the molecular size of the coupling sugars, also influence reactivity. The stereoselectivity of the glycosidation process may be controlled by the correct choice of reagents and conditions. The basic principles of oligosaccharide synthesis are shown in Fig 1.11.<sup>38</sup>

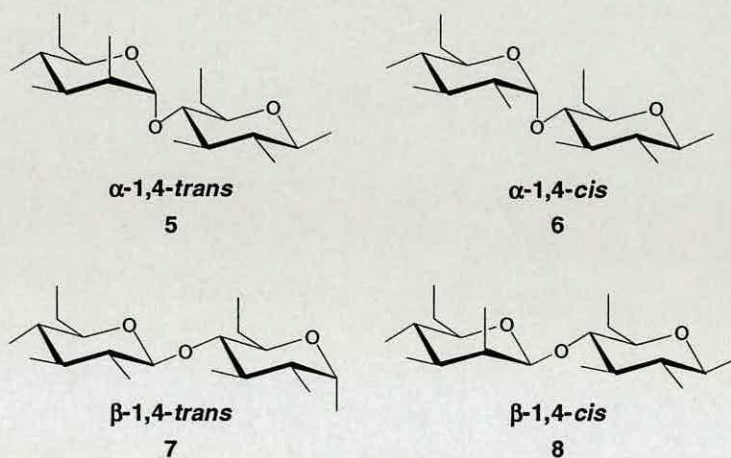




**Fig 1.11 :** *Coupling of a donor and an acceptor to generate a glycosidic linkage.*<sup>38</sup>

The donor molecule, 3, must be activated at the anomeric centre. Various activating groups and synthetic strategies have been used to achieve this, including halides (Koenigs-Knorr),<sup>64</sup> trichloroacetimidates<sup>63</sup> and thioalkyl moieties.<sup>65</sup> The acceptor molecule, 4, must be selectively protected at all but the reacting hydroxyl and this functionality must be reactive enough to enable coupling to occur.<sup>38</sup> Nucleophilic displacement of a leaving group at the anomeric position of the donor by the hydroxyl functionality of the acceptor molecule, 4, generates a disaccharide.<sup>38</sup>

The different types of 1,4-glycosidic linkage, with respect to the substituent at C-2, that can be formed during a chemical glycosylation reaction are illustrated in Fig 1.12.

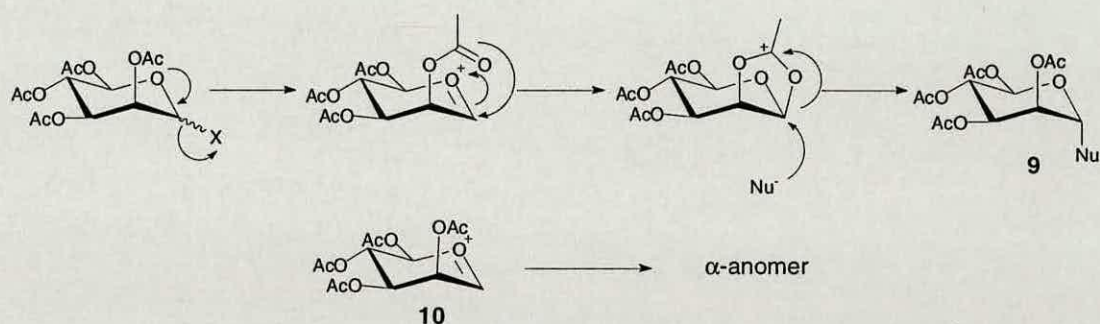


**Fig 1.12 :** *The different stereochemical results of glycosylation reactions.*

$\beta$ -1,4-*cis*-linked mannose, **8**, is an integral part of the pentasaccharide core common to all *N*-glycan species. As our research has been focussed on the synthesis of *N*-linked glycans some of the means used in the construction of  $\beta$ -1,2-*cis*-mannosyl linkages are of interest and will therefore be discussed in detail.

### 1.3.1. Chemical synthesis of $\beta$ -1,2-*cis*-mannosyl linkages.

$\beta$ -1,4-*cis*-mannosyl linkages are notoriously difficult to synthesise. As can be seen in Fig 1.13, the location of an axial acyl protecting group at C-2 in mannose that can participate in the glycosidation reaction (neighbouring group participation) leads predominantly to the  $\alpha$ -mannoside, **9**.<sup>66</sup>



**Fig 1.13 :** Neighbouring group participation in mannose sugars and oxonium ion, **10**, both give the  $\alpha$ -anomer.<sup>66</sup>

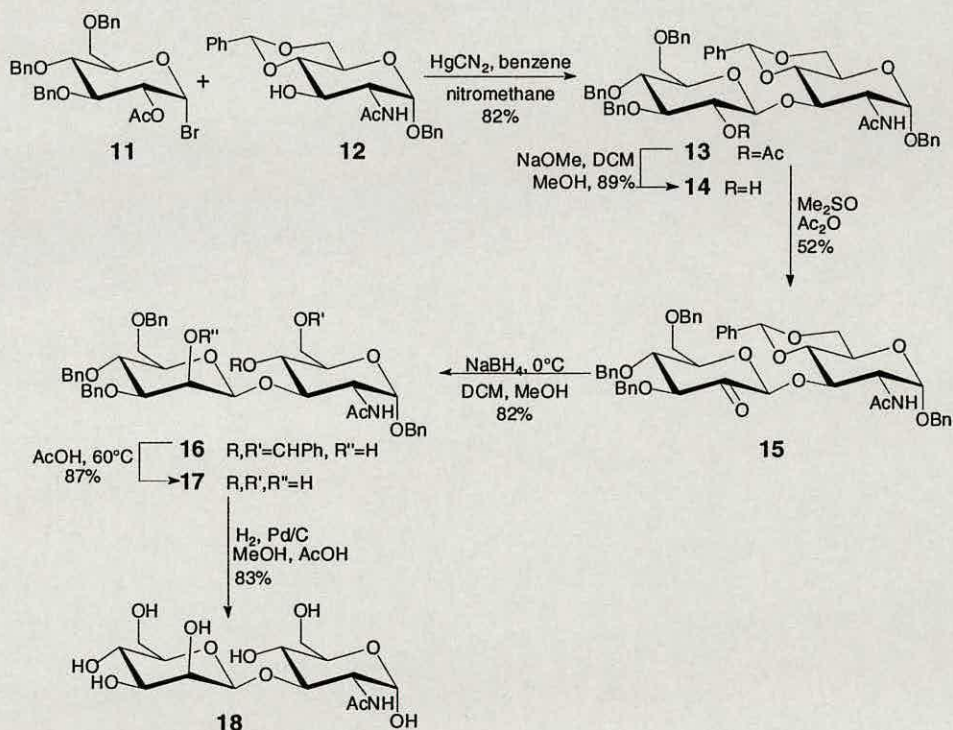
The anomeric effect also favours the formation of the  $\alpha$ -mannoside over the  $\beta$ -mannoside. The anomeric effect in pyranosides can be seen as the propensity of an electronegative substituent to adopt an axial orientation.<sup>66</sup> The axial conformer is stabilised by the  $\pi \rightarrow \sigma^*$  interaction of the axial orientated lone pair of electrons of the ring oxygen, with the antibonding orbital of the anomeric carbon bonded to an electronegative group.<sup>66</sup> Thus  $\alpha$ -mannosides are thermodynamically and kinetically favoured, and are the major product when the oxonium ion **10** is an intermediate in the glycosylation reaction, Fig 1.13.<sup>66</sup>



A vast array of methodologies has been developed to overcome these problems so that  $\beta$ -1,4-*cis*-mannosyl linkages can be constructed by chemical means. In order to give an overview of the synthesis of  $\beta$ -mannosides, details of some of the earliest methods up to more modern applications will be discussed.

### 1.3.2. Epimerisation of $\beta$ -D-glucosides.

Manipulation of  $\beta$ -D-glucopyranosides, to indirectly give the desired  $\beta$ -D-mannopyranosides, by oxidation of 2-OH to a ketone followed by reduction was first investigated by Jeanloz *et al.*<sup>67</sup> Condensation of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl- $\alpha$ -D-glucopyranosyl bromide, **11**, with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy- $\alpha$ -D-glucopyranoside, **12**, in the presence of mercuric cyanide gave **13** in 82% yield (Fig 1.14).<sup>67</sup>



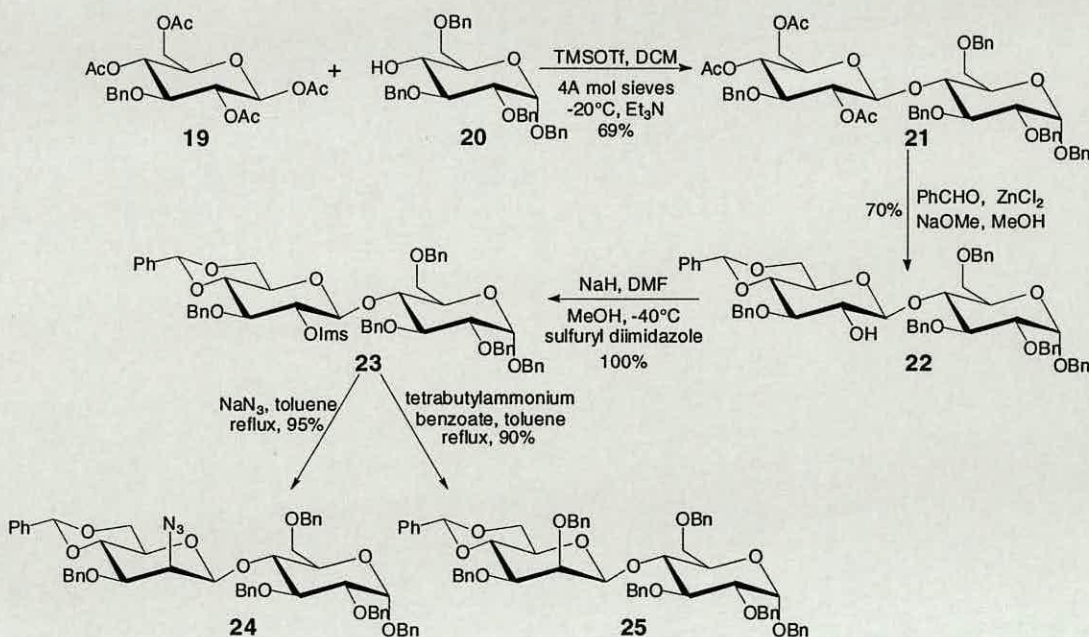
**Fig 1.14** : Key steps in the synthesis of 2-acetamido-2-deoxy-3-*O*- $\beta$ -D-mannopyranosyl-D-glucose.<sup>67</sup>

Following deacetylation of **13** to give **14**, oxidation with dimethyl sulfoxide-acetic anhydride gave benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-

benzyl- $\beta$ -D-arabino-hexopyranosyl-2-ulose)- $\alpha$ -D-glucopyranoside, **15**.<sup>67</sup>

Stereospecific reduction of the hexosidulose, **15**, with sodium borohydride gave the protected  $\beta$ -D-mannopyranosyl disaccharide **16**, which was partially hydrolysed to give **17**.<sup>67</sup> Hydrogenolysis of **17** in the presence of Pd/C gave the desired 2-acetamido-2-deoxy-3-O- $\beta$ -D-mannopyranosyl-D-glucose, **18**.<sup>67</sup>

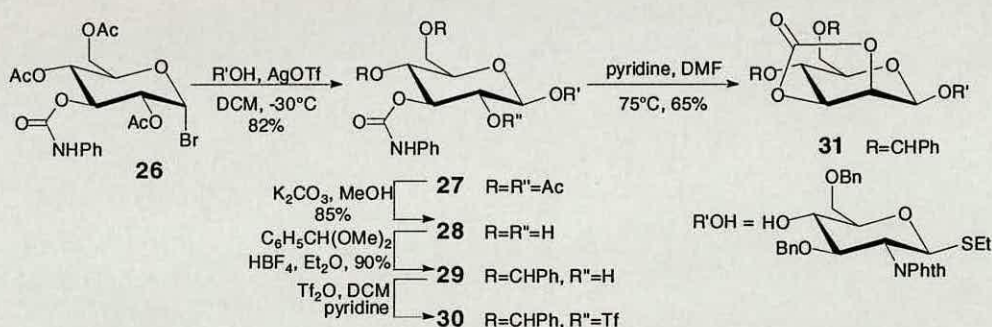
A modified version of this approach involved the use of an imidazylate in the synthesis of the  $\beta$ -mannosidic linkage.<sup>68</sup> 1,2,4,6-Tetra-O-acetyl-3-O-benzyl- $\beta$ -D-glucopyranose **19** was used as a precursor of the  $\beta$ -D-manno residue. Condensation of **19** with a sugar derivative partially protected with benzyl groups, **20**, in the presence of trimethylsilyl triflate as promotor, gave the  $\beta$ -D-glucopyranoside **21**, Fig 1.15.<sup>68</sup> Deacetylation of **21** followed by acetal protection of C4 and C6, and derivatisation with *N,N'*-sulfuryl diimidazole gave the imidazylate **23**.<sup>68</sup> Treatment with tetrabutylammonium benzoate or sodium azide in toluene at 80 °C, afforded the D-manno benzoate, **25**, or the 2-azido-2-deoxy-D-manno derivative, **24**, respectively.<sup>68</sup>



**Fig 1.15 :** Preparation of oligosaccharides with  $\beta$ -D-mannopyranosyl and 2-azido-2-deoxy- $\beta$ -D-mannopyranosyl residues.<sup>68</sup>



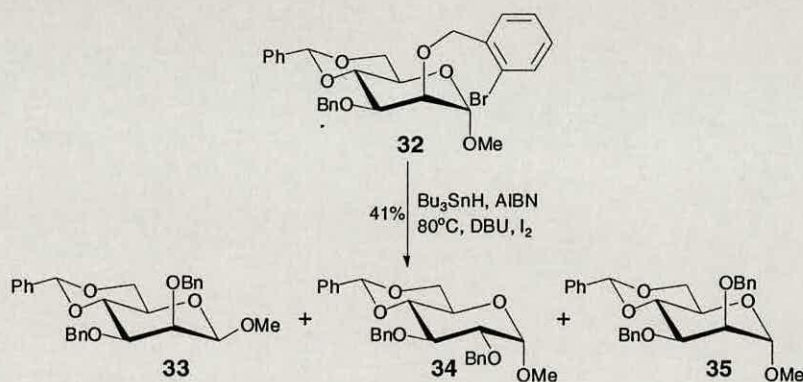
Another method by which  $\beta$ -mannosides can be obtained from the corresponding  $\beta$ -glucosides, *via* an inversion of configuration at C-2, is by the introduction of the oxygen nucleophile intramolecularly, in the sense of a neighbouring group effect.<sup>69</sup> The neighbouring group was introduced by formation of the 3-*N*-phenylurethane derivative of 1,2,5,6-di-*O*-isopropylidene- $\alpha$ -D-glucufuranose to generate the glycosyl donor, **26**. Glycosylation of **26** with a sugar ( $R'OH$ ) in the presence of silver triflate resulted in the formation of the  $\beta$ -glucoside **27**, Fig 1.16.<sup>69</sup> The first step in transforming the  $\beta$ -gluco compound **27**, into the  $\beta$ -manno compound **31**, required removal of the *O*-acetyl groups with  $K_2CO_3$  in methanol. The resulting sugar **28** was protected at positions C4 and C6 using a benzylidene acetal to give **29**.<sup>69</sup> The free 2-OH of  $\beta$ -glucoside **29** was activated with trifluoromethanesulfonic anhydride and pyridine for the inversion of configuration to give an insoluble triflate **30**, which was converted directly into  $\beta$ -mannoside **31** by heating to 75°C in pyridine/DMF.<sup>69</sup> The inversion at C-2 proceeded smoothly and without side-reactions because the intramolecular attack of the carbamoyl oxygen is favoured entropically.<sup>69</sup>



**Fig 1.16 :**  $\beta$ -mannosides from  $\beta$ -glucosides by intramolecular nucleophilic substitution.<sup>69</sup>

Anomeric inversion of  $\alpha$ -mannopyranosides to  $\beta$  by a radical translocation process has also been studied.<sup>70</sup> In a standard procedure, a 0.05M solution of **32** was heated for 14h at 80°C with tributyltin hydride and 2,2'-azobisisobutyronitrile (AIBN). After workup with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and  $I_2$ , three products were separated by flash column chromatography (Fig 1.17).<sup>70</sup> Reduction of the *o*-bromobenzyl derivative **32** provided two inverted products, the desired  $\beta$ -mannoside

**33** and the  $\alpha$ -glucoside **34** as well as the directly reduced product **35**.<sup>70</sup> The  $\beta$ -mannose derivative **33** results from 1,6-hydrogen transfer followed by reduction of the anomeric radical with tributyltin hydride, while the  $\alpha$ -glucose derivative **34** results from 1,5-hydrogen transfer followed by reduction of the resulting radical at C-2.<sup>70</sup> The directly reduced product **35** is believed to arise from bimolecular reduction of the aryl radical with tin hydride.<sup>70</sup>



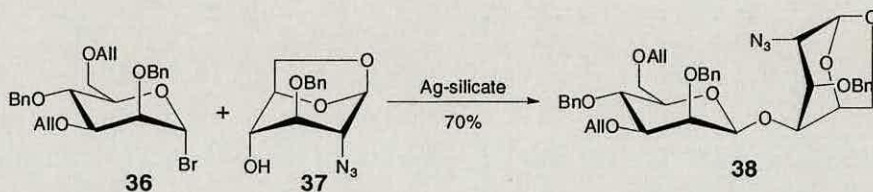
**Fig 1.17 :** Synthesis of  $\beta$ -mannopyranosides from  $\alpha$ -epimers by radical inversion.<sup>70</sup>

### 1.3.3. Heterogeneous catalysis.

A more direct method for producing the *cis*- $\beta$ -1,4-mannosidic linkage was developed by Paulsen where an insoluble silver catalyst was used to promote the conversion of the  $\alpha$ -halide to the  $\beta$ -glycoside.<sup>62</sup> When the reaction is carried out heterogenously, anomerisation to the reactive  $\beta$ -halide is severely restricted and reaction of the  $\alpha$ -halide with inversion takes precedence.<sup>64</sup> However, a halide with the greatest possible reactivity and a hydroxyl group of sufficient reactivity should be used, so that selectivity can be retained. With unreactive hydroxyl groups the proportion of  $\alpha$ -glycoside isolated was found to increase.<sup>64</sup>

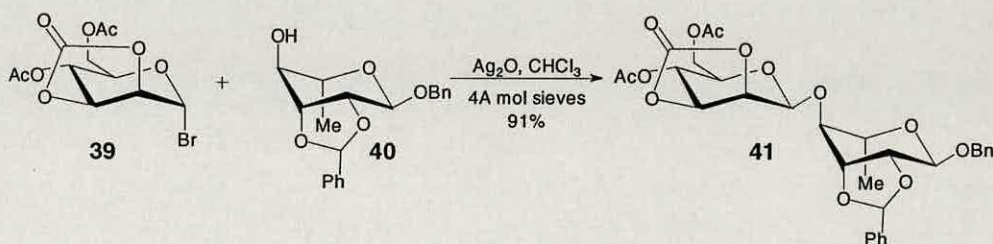
Donor **36** was activated at the anomeric centre by the presence of ether protecting substituents, while in acceptor **37**, the reactivity of the 4-OH group was improved by conversion of the gluco configuration into the 1,6-anhydro form.<sup>62</sup> Reaction of **36** and **37** in the presence of silver silicate afforded the desired  $\beta$ -linked disaccharide, **38**, in approximately 70% yield (Fig 1.18).<sup>62</sup>





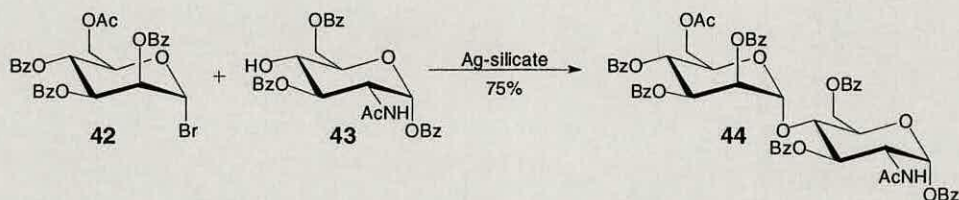
**Fig 1.18 :** Direct synthesis of the  $\beta$ -mannosidic linkage by heterogenous catalysis with silver silicate.<sup>62</sup>

The reaction of D-mannose derivatives with rhamnose derivatives in the presence of silver oxide and molecular sieves also produces the  $\beta$ -mannosidic linkage. This process was used in the synthesis of the pentasaccharide repeating unit of the O-specific polysaccharide from *Salmonella strasbourg*.<sup>71</sup> The key reaction involved glycosylation of benzyl 2,3-O-benzylidene- $\alpha$ -L-rhamnopyranoside, **40**, with mannosyl bromide **39** to give the disaccharide **41** in 90% yield (Fig 1.19).<sup>71</sup>



**Fig 1.19 :** Key step in the synthesis of the pentasaccharide repeating unit of the O-specific polysaccharide from *Salmonella strasbourg*.<sup>71</sup>

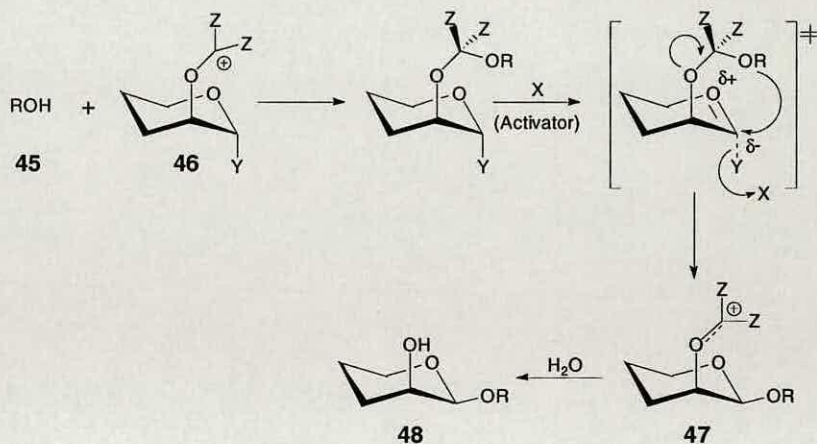
The success of this reaction may be attributed to the reactivity of the 4-OH group of the rhamnose derivative, which is comparable to that of a primary hydroxyl group.<sup>64</sup> When other secondary hydroxyl groups of moderate activity are used, the yield on this type of reaction is lowered considerably. For example, coupling of the reactive halide **42** with the glucosamine derivative **43** in the presence of a silver silicate catalyst, results in formation of the undesired  $\alpha$ -glycosidically linked product **44** in 75% yield (Fig 1.20).<sup>64</sup> The 4-OH group in **43** is known to have low reactivity and this has the effect of severely reducing selectivity in the direction of the  $\beta$  product.<sup>64</sup>



**Fig 1.20 :** *Coupling a reactive donor with a less reactive acceptor fails to produce  $\beta$ -glycosidically linked disaccharide.*<sup>64</sup>

#### 1.3.4. Intramolecular aglycon delivery.

Another strategy to overcome the problems associated with the construction of the 1,2-*cis*- $\beta$ -D-mannopyranosidic linkage uses intramolecular aglycon delivery to synthesise  $\beta$ -mannopyranosides.<sup>72</sup> The aglyconic alcohol, **45**, is covalently attached to a group on the O-2 position of a latent glycosyl donor, **46**, in a coupling reaction where stereospecificity is not a concern, Fig 1.21.<sup>72</sup> The aglycon is then delivered intramolecularly in a concerted reaction to produce the intermediate **47**, which, on quenching with water, gives the  $\beta$ -mannoside **48**. Quenching with other nucleophiles, for example benzyl alcohol, allows the synthesis of  $\beta$ -mannosides protected at O-2.<sup>72</sup>

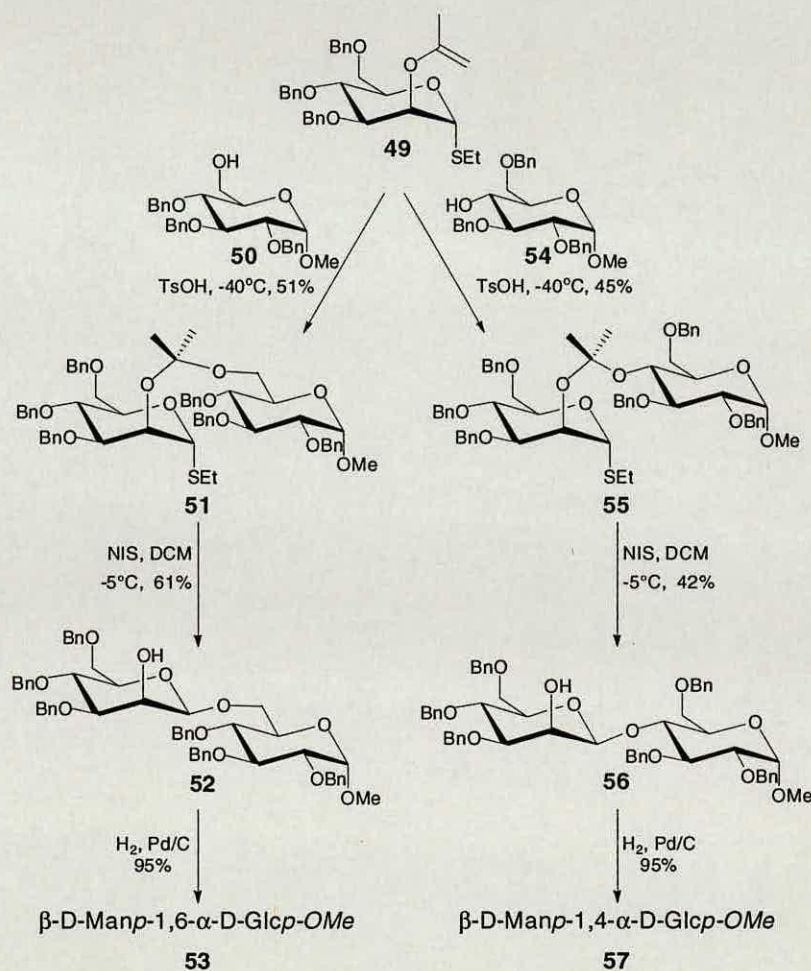


**Fig 1.21 :** *Intramolecular aglycon delivery.*<sup>72</sup>

There are many possibilities for the groups X, Y and Z. In the following example the activator, X, is *N*-iodosuccinimide, the substituent, Y, at the anomeric position is a thioethyl group while Z represents a methyl group. Treatment of vinyl ether **49** with



an equimolar amount of glycosyl acceptor **50** or **54** produced adduct **51** (51%) or **55** (45%) respectively (Fig 1.22).<sup>72</sup> Reaction of **51** or **55** with NIS in DCM gave the  $\beta$ -linked disaccharides (**52** or **56**) in 61% or 42% yield respectively, while no  $\alpha$ -linked disaccharides could be detected. Further transformation of **52** and **56** resulted in formation of the deprotected disaccharides **53** and **57** respectively.<sup>72</sup>

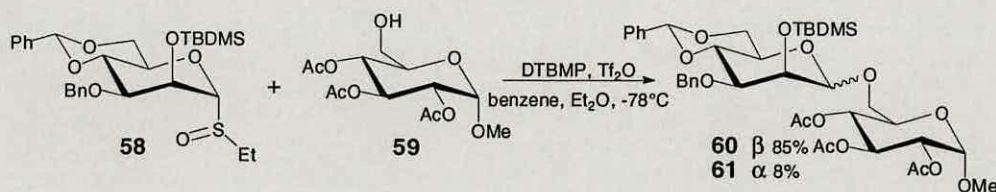


**Fig 1.22 :** *Synthesis of  $\beta$ -linked disaccharides by intramolecular aglycon delivery.*<sup>72</sup>

### 1.3.5. Formation of $\beta$ -mannose linkages using glycosyl triflates and glycosyl phosphates.

A protocol for the synthesis of  $\beta$ -mannosidic linkages based on sulfoxide glycosylation was developed by Crich and Sun.<sup>73</sup> Activation of sulfoxide **58** with Tf<sub>2</sub>O at -78°C, followed by addition of the acceptor, **59**, afforded  $\beta$ -mannoside **60** in

85% yield, together with only a minor amount of the corresponding  $\alpha$ -anomer **61** (8%) (Fig 1.23).<sup>73</sup>



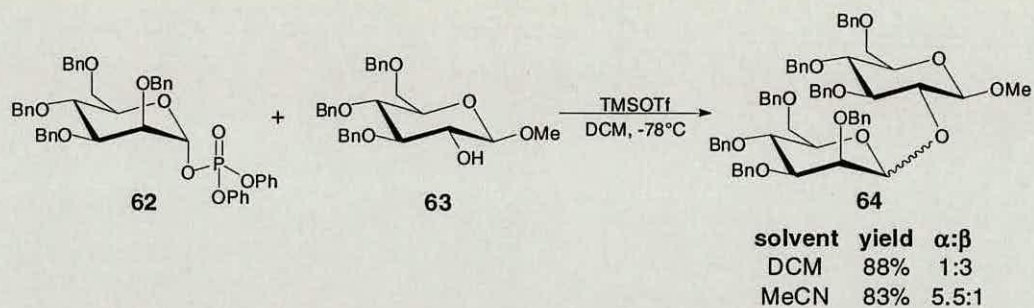
**Fig 1.23 : Synthesis of  $\beta$ -mannopyranosides via glycosyl triflates.**<sup>73</sup>

Glycosylation of **58** with a range of glycosyl acceptors in this way was investigated with  $\beta$ : $\alpha$  ratios as high as >20:1. Enhanced  $\beta$ -selectivity and improved yields were observed when the steric bulk of the *O*-2 protecting group of the acceptor was reduced and the coupling reaction was performed in a less ionising solvent than ether, for example DCM.<sup>73</sup>

More recently, glycosyl phosphates were found to show high  $\beta$ -selectivity in glycosylation reactions, even when a non-participating group was present in the C-2 position.<sup>74</sup> Activation of tetra-*O*-benzyl mannosyl phosphate **62** by TMSOTf at -78°C resulted in rapid glycosylation of the hindered acceptor **63**, to furnish disaccharide **64**, Fig 1.24.<sup>75</sup> The selectivity of this reaction was strongly dependent on the nature of the glycosyl acceptor and the solvent used for the coupling reaction. In DCM, the desired  $\beta$ -mannoside **64- $\beta$**  was preferentially formed, but when the less hindered C-6 hydroxyl group of galactose served as an acceptor, the  $\alpha$ -linked disaccharide was obtained as the main product.<sup>75</sup>

When the coupling reaction was carried out in acetonitrile, a reversal in selectivity was observed.<sup>75</sup> For the example shown, the disaccharide **64- $\alpha$**  was the main product obtained under these conditions from reaction of donor **62** with acceptor **63**. Even for the coupling of the less hindered galactose acceptor, enhanced  $\alpha$ -selectivity was observed in acetonitrile.<sup>75</sup>





**Fig 1.24 :** Formation of  $\beta$ -mannose linkages using glycosyl phosphates.<sup>75</sup>

Although the syntheses outlined above show great ingenuity and planning in achieving the target oligosaccharides, they have the considerable drawbacks of requiring complicated protection and deprotection strategies and activating groups. A mixture of anomers is quite often obtained and this causes the additional problem of separating  $\alpha$ - and  $\beta$ -linked oligosaccharides, which can be difficult.

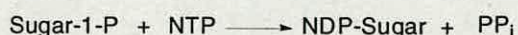
## 1.4. Chemoenzymatic synthesis of oligosaccharides.

In view of the inherent problems associated with the chemical synthesis of oligosaccharides, a more practical approach may be the use of chemoenzymatic methodologies. The high regio- and stereoselectivity and mild reaction conditions offered by enzyme-catalysed procedures provides an opportunity to tackle many synthetic problems encountered in carbohydrate synthesis. Enzymatic oligosaccharide synthesis can also decrease the length and complexity of a synthetic route by eliminating the need for complicated protected strategies.<sup>76</sup>

For *in vitro* enzymatic oligosaccharide synthesis three strategies are available. The first is the use of the glycosyltransferase enzymes of the Leloir pathway, which require sugar nucleotides as donors.<sup>77</sup> The second is the use of the non-Leloir pathway enzymes, which require sugar-1-phosphates as donors.<sup>76</sup> The third is the use of glycosidase or glycosyl hydrolase-catalysed reactions. Glycosidases usually cleave glycosidic bonds. However, studies have found that these enzymes can also be used for the formation of glycosidic bonds in a kinetic or thermodynamic approach.<sup>78</sup>

### 1.4.1. The Leloir pathway.

Three fundamental steps constitute the Leloir pathway *in vivo*: Activation, transfer and modification.<sup>78</sup> In the first steps of the Leloir pathway for all sugars, except NeuAc and its derivatives, a sugar (for example *N*-acetylglucosamine, glucose, galactose or mannose) is transformed into the corresponding sugar-1-phosphate by a kinase.<sup>78</sup> This sugar-1-phosphate reacts with a nucleoside triphosphate (NTP) in an enzyme-catalysed reaction and forms a chemically activated nucleoside diphosphate sugar (NDP-sugar) such as UDP-Glc, UDP-GlcNAc, UDP-Gal or UDP-Man.<sup>78</sup> The enzymes which catalyse such a process are known as pyrophosphorylases, or nucleoside transferases (Fig 1.25).<sup>78</sup>



**Fig 1.25 :** Formation of a chemically activated nucleoside diphosphate sugar.<sup>78</sup>



The activation of sialic acid (NeuAc) is an exception, as in this case a nucleoside monophosphate sugar forms directly from NeuAc itself (Fig 1.26).<sup>78</sup>



**Fig 1.26 : Direct formation of activated sialic acid.**<sup>78</sup>

Once the activated sugars have been formed, they are transferred to a protein or lipid, or to the non-reducing end of a growing oligosaccharide.<sup>78</sup> The enzymes which catalyse the transfer of an NDP-sugar are known as glycosyltransferases. For *N*-linked glycoproteins, these steps occur in the RER and in the Golgi apparatus.<sup>78</sup>

The final stage in the Leloir pathway involves modification of the substrate. For *N*-linked glycoproteins this modification occurs in two stages: The asparagine-linked tetradecasaccharide, Glc<sub>3</sub>-Man<sub>9</sub>-GlcNAc<sub>2</sub>, is firstly processed in a series of glycosidase-catalysed reactions which cleave the three terminal glucose and four mannose residues.<sup>5</sup> The exposed inner core, Man<sub>5</sub>-GlcNAc<sub>2</sub>, is then elaborated by the transfer of sugars from NDP-sugar species under the influence of glycosyltransferase enzymes.<sup>50</sup>

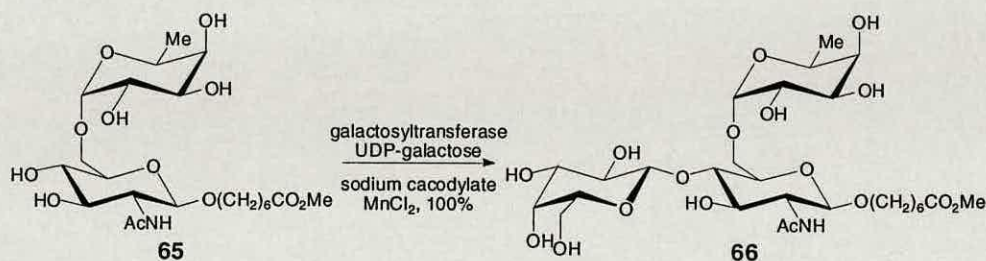
The *in vitro* synthesis of oligosaccharides depends on the availability of sugar nucleoside phosphates and glycosyltransferases. All of the common NDP-sugars are available from commercial sources, but most are expensive. Many glycosyltransferases are difficult to isolate, especially from mammalian sources, because the proteins are present in low concentrations and are membrane-bound.<sup>78</sup> If the enzyme is not readily available in sufficient quantities, the scale of the reaction is severely limited.

#### **1.4.2. The use of enzymes from the Leloir pathway in chemoenzymatic synthesis.**

The synthetic utility of many glycosyltransferases has been investigated and this has allowed the preparation and isolation of well-characterised oligosaccharides. One example of a combined approach using both chemical and enzymatic synthesis for

the preparation of a variety of sialyloligosaccharides representing the terminal sequences in oligosaccharides of glycoproteins and glycolipids has been reported by Sabesan and Paulson.<sup>79</sup> Neutral oligosaccharides prepared by chemical synthesis were sialylated enzymatically with purified mammalian sialyltransferase to form the  $\alpha$ -D-NeuAc-(2,6)- $\beta$ -D-Gal or  $\alpha$ -D-NeuAc-(2,3)- $\beta$ -D-Gal linkages.<sup>79</sup>

A number of groups have focused in particular on the study of galactosyltransferases. For example, Palcic *et al.* found that bovine D-galactosyltransferase transferred D-galactose from UDP-Gal to 6-O-substituted 2-acetamido-2-deoxy- $\beta$ -D-glucopyranosides.<sup>80</sup> The product of galactosylation of **65**, namely, 8-methoxycarbonyloctyl-2-acetamido-2-deoxy-6-O- $\alpha$ -L-fucopyranosyl-4-O- $\beta$ -D-galactopyranosyl- $\beta$ -D-glucopyranoside, **66**, was conveniently isolated from a preparative scale reaction by virtue of its hydrophobic linking-arm using absorption onto a reverse phase support and elution with 1:1 MeOH:water (Fig 1.27).<sup>80</sup>

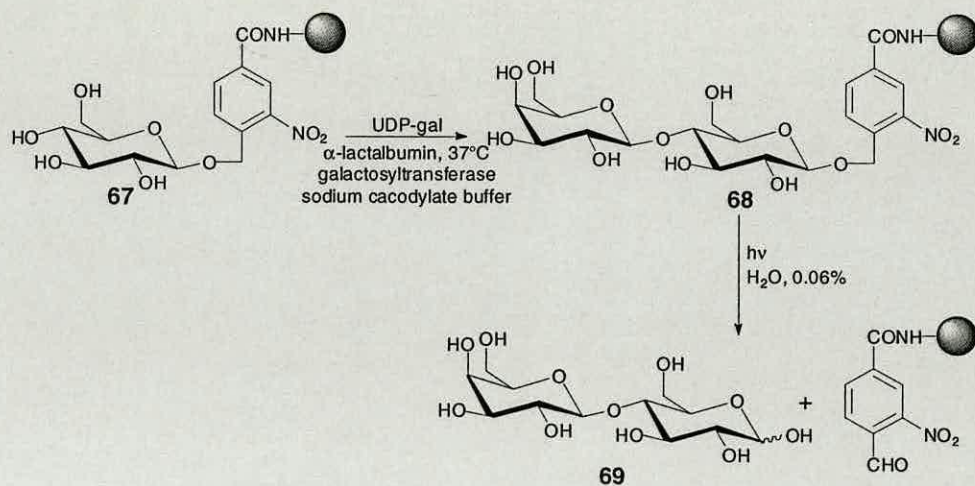


**Fig 1.27 :** Use of bovine D-galactosyltransferase to transfer D-galactose from UDP-Gal to 6-O-substituted 2-acetamido-2-deoxy- $\beta$ -D-glucopyranosides.<sup>80</sup>

Such reactions of glycosyltransferases have the potential for the rapid production of milligram quantities of oligosaccharides for use in biological testing, especially where synthetic oligosaccharide substrates are unavailable.<sup>80</sup>

Zehavi *et al.* used  $\beta$ -1,4-galactosyltransferases for their polymer-supported oligosaccharide synthesis (Fig 1.28).<sup>81</sup>





**Fig 1.28** : Use of  $\beta$ -1,4-galactosyltransferases for polymer-supported oligosaccharide synthesis.<sup>81</sup>

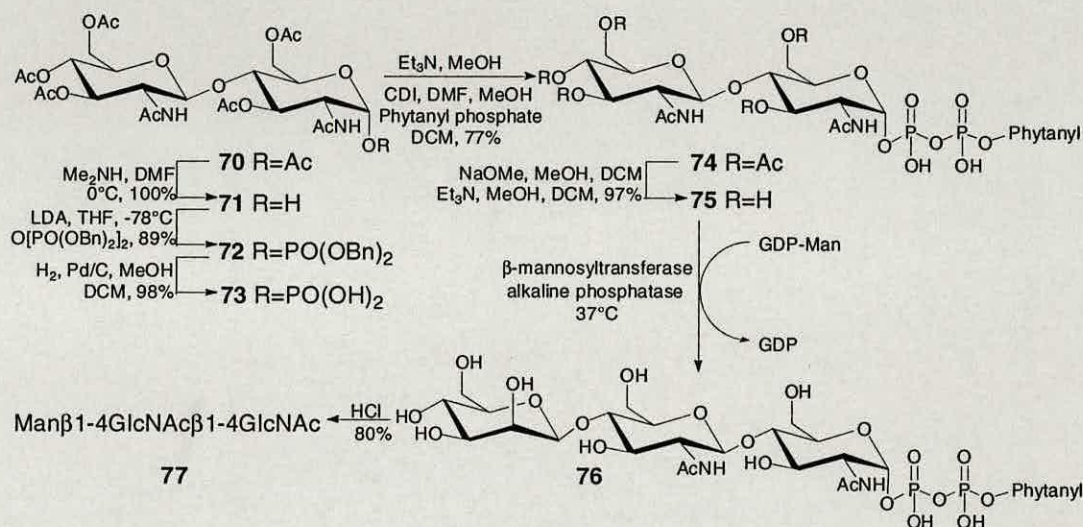
A glucose residue was attached to the polymer via a photocleavable 4-(*N*-polymer-amidocarbonyl)-2-nitrobenzyl linker to give solid-supported glucoside **67**.<sup>81</sup> The galactose moiety was transferred to glucoside **67** using  $\beta$ -1,4-galactosyltransferase and UDP-Gal to generate **68**. Finally, irradiation of **68** released the lactose moiety, **69**, from the polymer.<sup>81</sup>

The chemoenzymatic synthesis of oligosaccharides using recombinant glycosyltransferases has also been successfully accomplished.

The use of a combination of chemical and enzymatic techniques to synthesise the core trisaccharide  $\beta$ -D-mannopyranosyl-(1,4)-2-acetamido-2-deoxy- $\alpha$ -D-glucopyranose, **77**, both regio- and stereoselectively in good yield, has been reported by Flitsch *et al.*<sup>82</sup> A phytanyl-linked acceptor substrate **75** was prepared from commercially available chitobiose octaacetate, **70** (Fig 1.29). The chitobiose octaacetate was subjected to a selective anomeric deacetylation and then phosphorylated to give **72**.<sup>82</sup> Coupling of the deprotected chitobiose phosphate, **73**, with phytanyl phosphate in the presence of *N,N'*-carbonyl diimidazole (CDI), followed by deacetylation yielded the acceptor substrate **75**.<sup>82</sup> Incubation of the phytanyl-linked acceptor substrate **75** with GDP-mannose at 37°C in the presence of



the immobilised  $\beta$ -mannosyltransferase and alkaline phosphatase gave the phytanyl-linked trisaccharide **76**, which was subjected to acid hydrolysis without isolation.<sup>82</sup> Extraction of the cleaved lipids with chloroform was followed by gel-permeation chromatography of the aqueous phase to give the  $\beta$ -mannosyl-linked trisaccharide **77** in 80% yield.<sup>82</sup>



**Fig 1.29** : Synthesis of the core trisaccharide of N-linked oligosaccharides.<sup>82</sup>

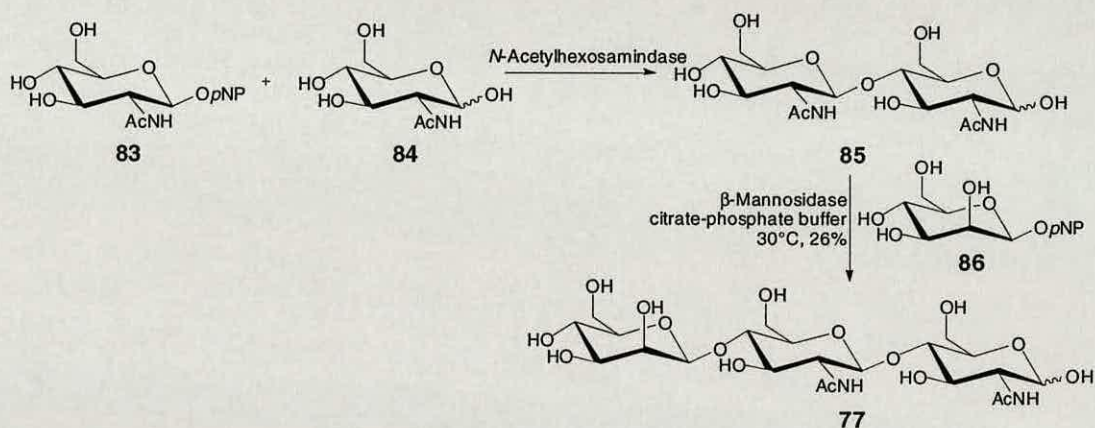
#### 1.4.3. The use of glycosidase enzymes in chemoenzymatic synthesis.

Glycosidases are the enzymes responsible for the hydrolytic cleavage of glycosidic bonds *in vivo*. Two groups of glycosidases exist: The exoglycosidases, which cleave terminal glycosidic units, and the endoglycosidases, which cleave both terminal and non-terminal glycosidic linkages in oligo- and polysaccharide chains.<sup>78</sup> Additionally, there are retaining glycosidases that preserve the stereochemistry of the anomeric centre of the glycoside and inverting glycosidases that invert this centre.<sup>76</sup>

The advantage of utilising glycosidases instead of glycosyltransferases as catalysts for glycosyl transfer is that expensive sugar nucleoside donors are not required to achieve reaction. The glycosidases are generally more available and less expensive than the glycosyltransferases.<sup>76</sup>



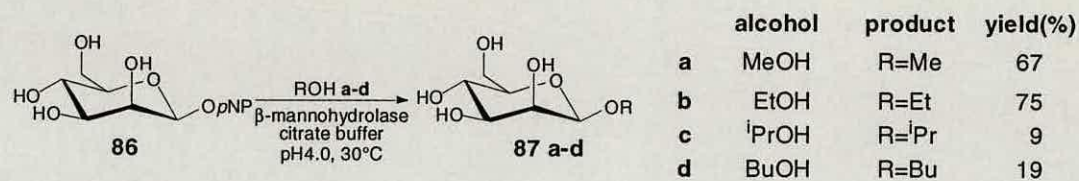
A two step synthesis of the core trisaccharide of *N*-linked glycoproteins using  $\beta$ -*N*-acetylhexosaminidase and  $\beta$ -mannosidase from *Aspergillus oryzae* has been accomplished by Singh *et al.* (Fig 1.30).<sup>83</sup> A one step procedure for the preparation of di-*N*-acetylchitobiose, **85**, was achieved by coupling the glycosyl donor *p*-nitrophenyl 2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside, **83**, with the acceptor *N*-acetylglucosamine, **84**, using a partially purified  $\beta$ -*N*-acetylhexosaminidase from *Aspergillus oryzae*.<sup>83</sup>



**Fig 1.30 :** Two step synthesis of the core trisaccharide of *N*-linked glycoproteins using  $\beta$ -*N*-acetylhexosaminidase and  $\beta$ -mannosidase from *Aspergillus oryzae*.<sup>83</sup>

A  $\beta$ -mannosidase was isolated from *Aspergillus oryzae* and used to catalyse the transfer of a  $\beta$ -mannosyl unit from *p*-nitrophenyl  $\beta$ -D-mannopyranoside, **86**, specifically to the 4-OH of the non-reducing unit of the disaccharide, **85**, to give the core trisaccharide, **77**, of *N*-linked glycoproteins.<sup>83</sup>

The enzymatic synthesis of alkyl and hydroxyalkyl  $\beta$ -D-mannopyranosides using a  $\beta$ -mannohydrolase enzyme from snails was undertaken by Taubken and Thiem.<sup>84</sup> Several  $\beta$ -mannopyranosides of water soluble alcohols were prepared by incubating 4-nitrophenyl  $\beta$ -mannopyranoside, **86**, with a high concentration of each alcohol in the presence of  $\beta$ -mannohydrolase at 30°C for several hours, Fig 1.31.<sup>84</sup> The resulting alkyl and hydroxyalkyl  $\beta$ -mannopyranosides **87a-d** were isolated in yields of up to 75%.<sup>84</sup>



**Fig 1.31 :** *The enzymatic synthesis of alkyl and hydroxylalkyl  $\beta$ -D-mannopyranosides using a  $\beta$ -mannohydrolase enzyme from snails.*

The major drawback in the use of enzymes as catalysts in carbohydrate synthesis involves the availability of the range of enzymes required to accomplish tasks in this area, and the stability and breadth in substrate acceptability of these enzymes. As genetic engineering progresses and cloning techniques develop, enzymes are becoming available in sufficient qualities and quantities, enabling greater feats to be achieved in oligosaccharide synthesis.



## **1.5. The lipid carrier for oligosaccharide assembly: dolichyl phosphate.**

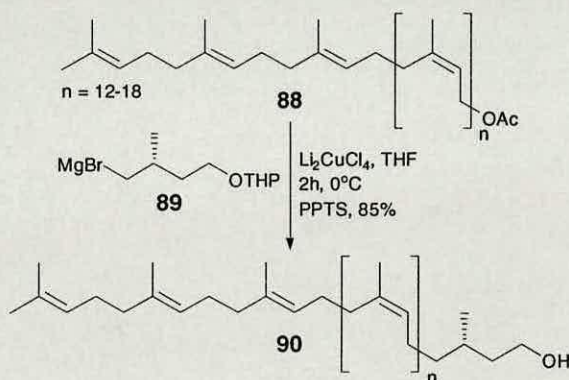
The polyisoprenoid dolichol is well established as being an essential cofactor in the biosynthesis of glycoconjugates.<sup>85</sup> The *N*-linked oligosaccharides of glycoproteins are first biosynthesised on dolichol before transfer to the nascent protein chain in the lumen of the ER.

By means of such hydrophobic vehicles the very hydrophilic glycoside chains can be attached to the specific target sites of the acceptor proteins in the hydrophobic membrane folds of the endoplasmic reticulum.<sup>86</sup> The glycosyltransferases involved in the biosynthesis of *N*-linked glycoproteins are all membrane associated and act on lipid substrates. The extent of molecular recognition of the dolichol lipid by these transferases is poorly understood.<sup>87</sup> The ability of the enzymes to recognise dolichols *in vivo* is assumed to reside in a portion of the protein in the membrane.<sup>88</sup> Indeed, comparison of the sequences of yeast glycosyltransferases, ALG7, ALG1 and DPM1, known to interact with Dol-P derivatives revealed that they shared a conserved 13-amino acid peptide sequence in obvious membrane-spanning regions of the proteins.<sup>89</sup>

### **1.5.1. The occurrence, isolation and synthesis of dolichol species.**

In order to effectively study the steps involved in the biosynthesis of lipid-linked oligosaccharides and the subsequent transfer of the oligosaccharide to an asparagine residue of a growing peptide in co-translational modification processes, it is essential to have a source of pure dolichols in quantities sufficient to allow synthesis of both biosynthetic intermediates and synthetic analogues in the pathways responsible for the biogenesis of glycoproteins. Supplies of naturally occurring dolichols are scarce, and can only be isolated from eukaryotic cells in trace quantities by arduous isolation procedures. Dolichols have been extracted from pig liver, but following a long and tedious procedure, a mere 200mg of a mixture of homologues was isolated from 5kg of pig liver.<sup>90</sup>

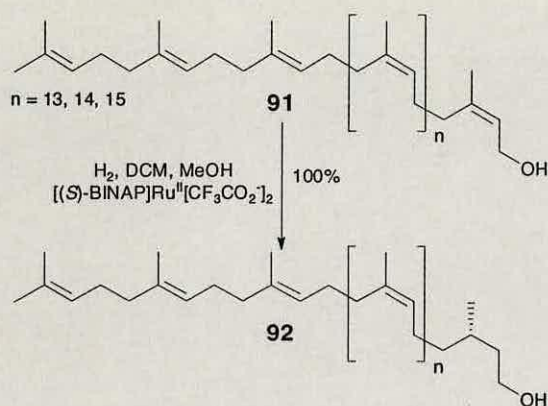
The first synthetic preparation of optically active dolichols, **90**, from a mixture of polyprenols isolated from the leaves of *Ginkgo biloba* was reported by Suzuki *et al.*<sup>91</sup> The synthesis was achieved by the addition of the optically active saturated isoprene unit, **89**, to a mixture of polyprenyl acetates, **88**, using a grignard coupling reaction, Fig 1.32.<sup>91</sup> The main disadvantage of this synthetic preparation was the need for a tedious HPLC resolution step in the synthesis of the optically active Grignard reagent, **89**.<sup>91</sup>



**Fig 1.32 :** The synthesis of mammalian dolichols from plant polyprenols.<sup>91</sup>

A second synthetic route to dolichol involved ruthenium-based homogeneous hydrogenation of naturally occurring polyprenols with  $\{(S)\text{-BINAP}\}\text{Ru}^{\text{II}}\{\text{CF}_3\text{CO}_2^-\}_2$ .<sup>92</sup> Again, the starting polyprenols, **91**, were extracted in the form of the corresponding acetates from the leaves of the *Ginkgo biloba* tree and then saponified.<sup>92</sup> Asymmetric hydrogenation using a Noyori catalyst afforded the desired (*S*)-dolichols, **93**, Fig 1.33.<sup>92</sup> The starting polyprenols had to be rigorously purified prior to saponification, to prevent traces of by-products poisoning the catalyst. Problems were also encountered with poor solubility of the purified polyprenols in the solvent medium of the reaction.





**Fig 1.33 :** *Synthesis of dolichols via asymmetric hydrogenation of plant polyprenols.*

Although dolichols can be synthesised, they are usually obtained as a mixture of homologues that are difficult to manipulate. Therefore, what is needed is a readily available synthetic analogue of dolichol, which is an acceptor for glycosyltransferases. This would allow for the isolation and purification of unidentified transferases involved in the pathways of *N*-glycan biosynthesis, as well as providing a novel route to the pentasaccharide core.

## 1.6. Conclusion.

Glycoproteins, along with glycolipids, are known to act as sites for the binding of other large biomolecules such as hormones, antibodies, enzymes, bacterial toxins and cell surface proteins. The impact of protein glycosylation can be seen in the important physical properties imparted to the molecule, for example, protease resistance, conformational stability, charge and water-binding capacity.<sup>66</sup> The number of monosaccharides and the possible ways of linking them together gives the glycal portion of glycoproteins immense diversity in structure compared with the protein, implicating them as the key elements in the complex subtleties involved in physiological and pathological processes of living organisms.<sup>66</sup>

*N*-Linked glycoproteins originate from the biosynthetic precursor dolichol phosphate-linked glycal, Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub>-PP-Dol, in the ER of eukaryotic cells. Yeast genetic techniques have helped to identify many of the enzymes required for the stepwise assembly of Glc<sub>3</sub>Man<sub>9</sub>GlcNAc<sub>2</sub>-PP-Dol, yet some of those enzymes involved in the early stages of *N*-glycan biosynthesis remain unidentified. In order to gain more information about the unknown mannosyltransferases that are active in the early stages of *N*-glycan biosynthesis, a phytanyl-linked monosaccharide (glucosamine), **163**, and disaccharide (chitobiose), **75**, were prepared. These substrates should prove useful for providing biosynthetic intermediates for the studies of subsequent glycosyltransferases of the biosynthetic pathway of *N*-linked oligosaccharides.

In order to effectively study the steps involved in the biosynthesis of lipid-linked oligosaccharides and their subsequent transfer to a nascent protein in co-translational modification processes, it was also essential to have a source of pure dolichols in sufficient quantities to allow the synthesis of both biosynthetic intermediates and synthetic analogues in the pathways responsible for the biogenesis of glycoproteins. Since supplies of naturally occurring dolichols are scarce and as they are difficult to synthesise, a number of lipid substrates were prepared instead as synthetic analogues of the naturally occurring dolichyl phosphate.



The aim of this project was to synthesise three novel analogues of the naturally occurring lipid, dolichyl phosphate (Dol-P). All of these lipids were designed to be multi-functionalised. An ester substituent was incorporated into the backbone of the lipid chain in order to increase their solubilities in water. Two of the lipids had an *N*-phthalimido group at the  $\omega$ -end of the chain, while the third bore a fluorescent probe at this point. The requirements for chain length and polyisoprenoid structure were then to be tested. It was reasoned that the fluorescent label would be of some aid in characterising the interactions of Dol-P or its glycosylated derivatives with enzymes which utilise these substrates or proteins participating in sugar transport across the cell membrane.

## 2. Synthesis of Novel Lipid Analogues of Dolichyl Phosphate.

### 2.1. Introduction to past research.

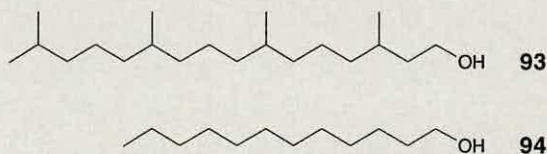
A possible function of dolichol is to act as a lipophilic membrane anchor, and it was thought that this property could be effectively mimicked by more readily available and easily synthesised isoprenoid substrates. Early research has found that certain restrictions apply to the selection of suitable dolichol analogues.<sup>93</sup> A study of the formation of dolichol diphosphate-(*N*-acetylglucosamine)<sub>1-2</sub>, (Dol-PP-(GlcNAc)<sub>1-2</sub>) from uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc) and dolichyl monophosphate (Dol-P) revealed an obligatory requirement for  $\alpha$ -saturated polyprenols.<sup>93</sup> In addition, the substrate specificity of solubilised enzymes responsible for (1) the transfer of the chitobiosyl group from Dol-PP-(GlcNAc)<sub>2</sub> to a hexapeptide containing an Asn-Leu-Thr-Ser sequence, and (2) the transfer of mannose from guanosine diphosphate mannose (GDP-Man) to Dol-P and from Dol-P-Man to a threonine-containing tetrapeptide have been investigated.<sup>93</sup> The activity of all the enzymes concerned depended strongly on the saturation of the  $\alpha$ -isoprene unit and, furthermore, clear chain-length effects were also observed.<sup>93</sup> For the ALG7 protein the Michaelis constant,  $K_M$ , for Dol-P was found to be 31  $\mu$ M.<sup>89</sup> The Michaelis constant is defined as the substrate concentration in moles/L at which an enzyme-catalysed reaction proceeds at half its maximal rate.<sup>94</sup> As the isoprenoid chain length was decreased from 100 to 55 carbons, the  $K_M$  increased to 117  $\mu$ M while an isoprenoid derivative with only 35 carbons gave rise to very low activity in the assay.<sup>89</sup> For the DPM1 protein, the  $K_M$  for Dol-P was approximately 1  $\mu$ M. Decreasing the isoprenoid length to 35 carbons decreased the reaction velocity by 10-fold.<sup>89</sup> From this study it was concluded that a minimal chain length of seven isoprene units is required based on observations that a polyprenol phosphate of this size was either inactive or showed only about one tenth of the donor or acceptor specificity.<sup>89</sup>

Prior to this work, the fully saturated tetraprenyl phosphate, phytanyl phosphate, had been reported by Clark and Villemez to be an efficient analogue of dolichyl



phosphate in the enzyme mediated formation of the monosaccharide lipid phosphate Dol-P-Man using a mannosyl transferase from mung beans.<sup>95</sup> On the basis of this information, phytanol, **93**, appeared to Taylor *et al.* to be a good starting point in the search for novel analogues of the natural lipid dolichol, since it is readily available in gram quantities from phytol by catalytic hydrogenation and it possesses the  $\alpha$ -saturated isoprene unit necessary for retention of mannosyl transferase activity.<sup>35</sup> Given the previous observations on the effect of chain length,<sup>93</sup> Taylor *et al.* hoped that the requirement for a dolichol chain of at least seven isoprene units would become less rigorous as the length of the oligosaccharide chain increased.<sup>35</sup> Taylor *et al.* also anticipated that phytanol would be sufficiently lipophilic to be retained at the membrane surface and that it would be held within the dolichol binding site of the mannosyl transferase.<sup>38</sup>

The simple, straight chain lauryl alcohol, **94**, was also chosen by Taylor *et al.* to replace the natural dolichyl lipid.<sup>35</sup> Although lauryl alcohol possesses none of the previously discussed structural requirements for transferase activity, it could provide useful information on the nature of the dolichol binding site.<sup>38</sup> Both of these lipids are illustrated in Fig 2.1.

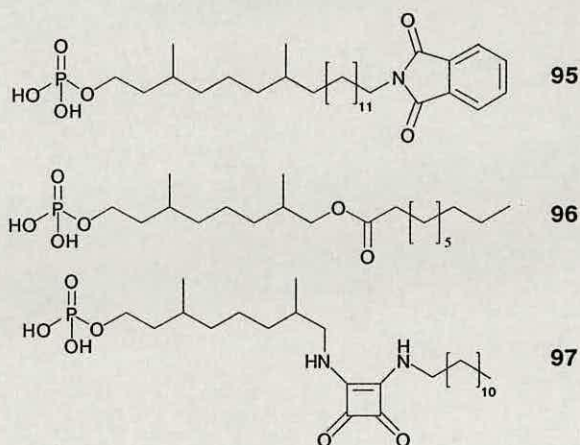


**Fig 2.1 :** *Phytanol and lauryl alcohol, the first two lipids chosen as dolichol analogues in the group.*<sup>38</sup>

Biological assays of these two lipid phosphates showed that two mannosyl transferases in crude extracts of pig liver and yeast accepted phytanol analogues of dolichol-linked acceptor substrates.<sup>35</sup> The fact that the branched phytanol, **93**, but not the straight chain lauryl alcohol, **94**, could act as a substitute for dolichol revealed the requirement for methyl branching in any new dolichol analogue.<sup>35</sup> Since the structural feature common to both dolichyl phosphate and phytanyl phosphate is the

first saturated isoprene unit, it appeared that the methyl group nearest the phosphate headgroup was necessary for enzyme activity.<sup>35</sup>

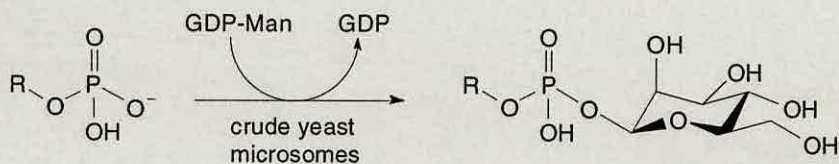
In order to gain a better understanding of the role of the polyisoprenoid structure in substrate recognition by the transferases involved in *N*-glycan biosynthesis, further studies within our group focused on the synthesis of a number of functionalised lipids.<sup>96</sup> These lipids conform to the restrictions required for analogues of dolichol in that they fall within the predicted limits for chain length, bear an  $\alpha$ -saturated isoprene unit and contain two methyl branches close to the phosphate headgroup. Heteroatom modification of the lipophilic chain was introduced to improve substrate solubility in water and also to test the tolerance of the dolichol binding site to heteroatom substituents.<sup>96</sup> The lipid structures investigated are illustrated in Fig 2.2.



**Fig 2.2 :** Multifunctionalised lipids synthesised to gain further information on the nature of the dolichyl binding site.<sup>96</sup>

Enzymatic assays of these three lipids were conducted using dolichyl phosphate mannose synthase (Dol-P-Man synthase), one of the transferases under investigation that had been found to accept phytanol derivatives<sup>35</sup> and to possess a putatively conserved dolichyl binding site.<sup>89</sup> Dol-P-Man synthase is an endoplasmic reticulum resident membrane enzyme that catalyses the transfer of mannose from GDP-Man to dolichyl phosphate, Fig 2.3. This results in the formation of a key glycosyl donor in pathways for the synthesis of *N*-linked oligosaccharides and *O*-linked oligosaccharides in yeast glycoproteins and glycosyl phosphatidylinositol anchors.<sup>97</sup>

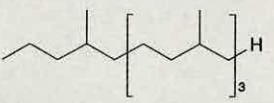
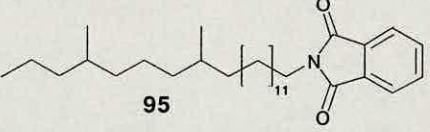
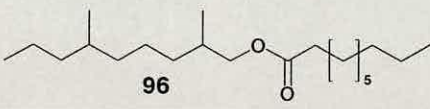
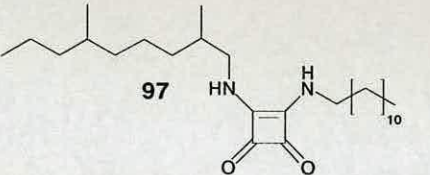




R is the lipid component attached to the phosphate headgroup.

**Fig 2.3 :** *The transfer of mannose from GDP-Man to a lipid phosphate as catalysed by the enzyme Dol-P-Man synthase. The different lipid components represented by R are illustrated in Table 1 below.*<sup>98</sup>

Each phospholipid was incubated in parallel with dolichyl phosphate, **2**, and phytanyl phosphate, **98**, with a crude enzyme system from yeast in the presence of a <sup>14</sup>C-labelled GDP mannose co-factor.<sup>98</sup> The relative levels of transfer of the radiolabelled mannose to the lipid were determined by scintillation counting of an organic extract of the reaction.<sup>98</sup> The enzymatic assay with Dol-P-Man synthase showed different levels of activity for the transfer of radiolabelled mannose to the different phospholipids, Table 1.

R	Incorporation relative to dolichyl phosphate
Dolichol <b>2</b>	1.00
 <b>98</b>	0.62
 <b>95</b>	0.44
 <b>96</b>	0.60
 <b>97</b>	0.00
No exogenous acceptor	0.00

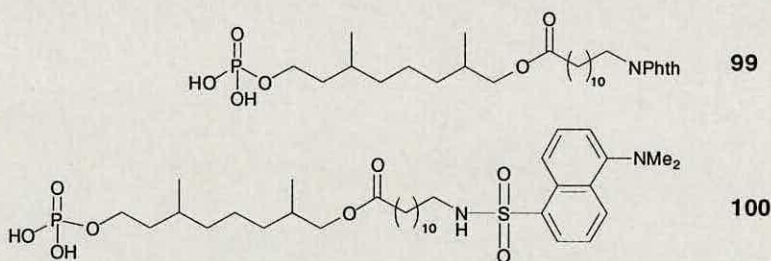
**Table 1.**<sup>96</sup>

The greatest transfer of radiolabelled mannose to a lipid was observed for dolichyl phosphate, **2**, and the activity for this process was set at 100%. Phytanyl phosphate, **98**, was found to be the best synthetic analogue showing 62% activity relative to dolichyl phosphate. The results in Table 1 show that the transfer of mannose was not observed in the presence of compound **97**.<sup>96</sup> This novel lipid phosphate was not found to be a suitable acceptor substrate for mannosyl transferase enzymes. This suggested that the structural variations in the lipid backbone of compound **97**, such as the incorporation of the bulky cyclobutadione and two amino groups, were not compatible with the dolichyl binding site.<sup>96</sup> However, the soluble acceptor lipids **95**<sup>98</sup> and **96**<sup>96</sup> compared well to phytanyl phosphate, **98**, as a substrate, showing 44% and 60% activity respectively, relative to dolichyl phosphate, **2**. From this, it appeared that the dolichyl binding site was tolerant of polar groups at the  $\omega$ -position and simple heteroatom modifications of the lipid chain.<sup>98</sup>



## 2.2. Synthesis of novel dolichyl analogues.

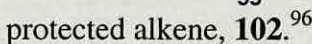
On the basis of these results, it was decided that our novel analogue for the natural dolichyl lipid would contain a combination of the structural features of the two active lipid substrates, **95** and **96**. The bi-functionalised molecule, **99**, retained two methyl branches close to the phosphate headgroup, an  $\alpha$ -saturated isoprene end unit while the chain length fell within predicted limits, Fig 2.4. The heteroatom modification of the lipid backbone would not only increase the solubility of the lipid substrate in water, but it also allowed for the possibility of extending the chain length of intermediate compounds, such as **106**, Fig 2.5. The flexibility of this synthetic strategy gave the ability to alter the functionality at the  $\omega$ -position to allow both attachment of the lipid to solid support or derivatisation of substrate mimics for specific glycosyltransferases. Furthermore, the synthesis of a novel analogue of dolichol with a fluorescent label at the  $\omega$ -end of the lipid chain would be of some aid in characterising the interactions of Dol-P or its glucosylated derivatives with enzymes which utilise these substrates or proteins participating in sugar transport across the membrane. The fluorescently labelled lipid, **100**, chosen as a novel derivative of dolichyl phosphate is also illustrated in Fig 2.4.



**Fig 2.4 :** *The two target lipids chosen as possible alternatives to dolichyl phosphate.*

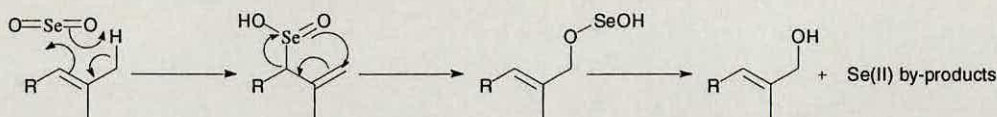
### 2.2.1. Synthesis of 3,7-dimethyl-8-(12-*N*-phthalimidododecanoyloxy)octyl phosphate.

The synthesis of lipid **99** began with benzyl protection of commercially available citronellol, **101**, as shown in Fig 2.5. Citronellol, **101**, was treated with sodium hydride in THF for one hour at room temperature to generate an alkoxy anion.



The next step involved oxidation of the allylic group of **102** using catalytic selenium dioxide as the oxidising agent. Selenium dioxide displays a unique mode of interaction with olefins, involving an initial ene reaction followed by a [2,3] sigmatropic rearrangement.<sup>99</sup> The initial product is an allylic seleninic acid which undergoes allylic rearrangement to give an unstable compound that rapidly decomposes to an allylic alcohol as illustrated in Fig 2.6.<sup>2</sup>





**Fig 2.6 :** Oxidation of an alkene to an allylic alcohol with  $\text{SeO}_2$ .<sup>2</sup>

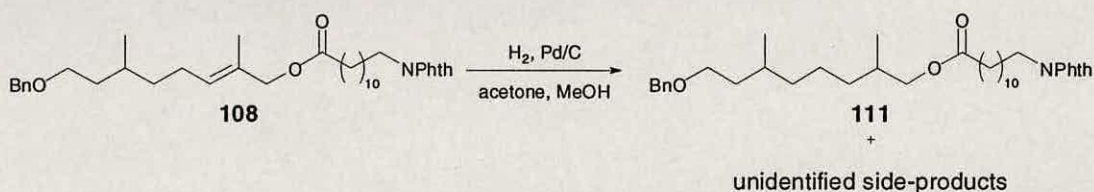
The utility of selenium dioxide as a selective allylic oxidant has been improved by the use of *tert*-butyl hydroperoxide, which as co-oxidant effectively prevents interference by reduced selenium species giving a cleaner oxidation.<sup>100</sup> *tert*-Butyl hydroperoxide re-oxides Se(II) species after the catalytic cycle, thereby preventing the formation of diols and epoxides and eliminating the need for disposal of large amounts of toxic selenium-containing products.<sup>100</sup>

The selenium-catalysed oxidation of acyclic olefin, **102**, produced, in addition to the desired allylic alcohol, **103**, an allylic aldehyde, **104**. The aldehyde side-product, **104**, was isolated in 23% yield by flash column chromatography, while the allylic alcohol, **103**, was recovered in 39% yield. In order to increase the amount of **103**, sodium borohydride mediated reduction of the  $\alpha,\beta$ -unsaturated aldehyde, **104**, was attempted in an ethanol-water solution.<sup>96</sup> This process did not proceed efficiently, giving a mixture of products which upon separation by flash-column-chromatography afforded the desired allylic alcohol, **103**, (28%) and the saturated alcohol (12%).

The synthesis of the key intermediate ester, **106**, was achieved in 87% yield by the addition of commercially available 12-bromododecanoic acid, **105**, 4-dimethylaminopyridine (DMAP) and dicyclohexylcarbodiimide (DCC) to a solution of the benzyl protected allylic alcohol, **103**, in anhydrous diethyl ether.<sup>101</sup> The flexibility of the synthetic strategy would allow esterification of the allylic alcohol, **103**, with carboxylic acid substrates of different chain lengths. It is also possible at this point in the synthetic route to alter the functionality of the heteroatom substituent in the lipid chain.

Introduction of a nitrogen-containing group at the  $\omega$ -position commenced by heating the brominated lipid, **106**, with potassium phthalimide, **107**, at 80 °C in DMF.<sup>96</sup> It was anticipated that the *N*-phthalimido lipid, **108**, obtained in 84% yield from this step, would fulfil a two-fold purpose. Firstly, it would be tested to establish enzyme activity for this type of multi-functionalised lipid and, secondly, it would be used as a synthetic intermediate in the synthesis of the fluorescently labelled dolichyl phosphate derivative, **100**, Fig 2.14 (section 2.2.2.).

The final steps in the synthesis of the target lipid **99** required attachment of the phosphate headgroup to the  $\alpha$ -saturated isoprene unit, Fig 2.5. It was hoped that unmasking of the benzyl protected alcohol and reduction of the double bond could be achieved in one-step by catalytic hydrogenation over finely divided 10% palladium on charcoal in 2:1 acetone:methanol. This reaction did not proceed as efficiently as anticipated with problems being encountered in the purification and isolation of the desired product. Partial hydrogenation was frequently encountered to give a product such as **111**, Fig 2.7, where loss of the double bond and retention of the benzyl protecting group was observed.



**Fig 2.7 :** *Partial hydrogenation resulted in loss of the double bond but gave a lipid which still retained the benzyl protecting group. A number of unidentified side-products were also produced in this reaction.*

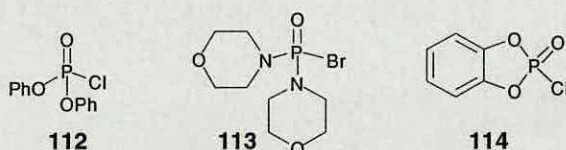
These problems may have been due in part to the low temperatures in the lab at the time this work was carried out. The pale yellow oil obtained after the reaction was worked-up would rapidly turn to a white residue, which was difficult to purify, and low yields of all products were obtained. Only when the lab temperature increased did the efficiency and yield of this process improve to generate the saturated product, **109**, as a mixture of diastereomers at positions 3 and 7 in 66% yield. Previous work



has indicated that stereochemistry at position 3 has a negligible effect on activity.<sup>98</sup> The phytanyl lipid used in previous experiments was also a diastereomeric mixture at positions 3 and 7 but it shows remarkably high activity (62%) compared to dolichyl phosphate.<sup>98</sup> It has thus been assumed for the scope of this project that the stereochemistry of the methyl groups on the lipid chain does not have a dramatic influence on the activity of the mannosyl transferase enzymes.

Phosphorylation of the alcohol **109** was the next step to be undertaken. Previously, phosphorylation of a lipid alcohol was carried out under conditions developed by Perich and Johns where treatment with *N,N'*-diisopropyl-di-*tert*-butyl phosphoramidite and 1-*H*-tetrazole generated a phosphite.<sup>102</sup> Oxidation of the crude phosphite with *meta*-chloroperbenzoic acid (mCPBA) would then produce the protected di-*tert*-butyl phosphate. At the time that this procedure was to be conducted, 1-*H*-tetrazole was not commercially available and so alternative methods were investigated.

The synthesis of monoalkyl phosphates has been reported by a number of people using a wide variety of phosphorylating reagents, some of the most common being illustrated in Fig 2.8.<sup>38</sup>

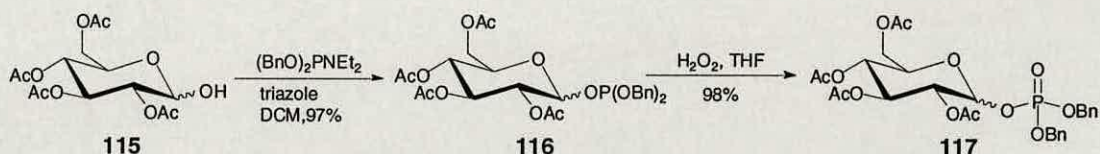


**Fig 2.8 :** Some common phosphorylating reagents used in the synthesis of monoalkyl phosphates.<sup>38</sup>

Diphenylphosphochloridate, **112**, has been shown to be much more stable and can be stored for several months.<sup>38</sup> However, the formation of diphenylphosphates can be problematic and removal of the phenyl groups can be quite a challenge.<sup>38</sup> Another unstable phosphorylating reagent that has been used is dimorpholinephosphorobromidate, **113**, which is readily prepared but unsuitable for storage.<sup>38</sup> The *o*-phenylenephosphorochloridate, **114**, has been successfully utilised

in the production of monoalkyl phosphates.<sup>38</sup> However, problems have been encountered in both the formation and deprotection of the resulting phosphotriesters, giving less than satisfactory yields.<sup>38</sup>

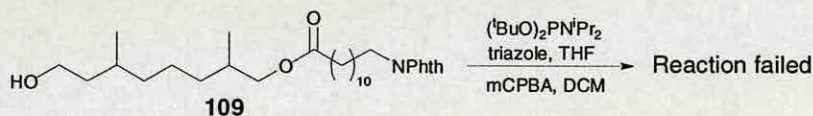
A similar method to that of Perich and Johns had been reported by Wong *et al.* utilising dibenzyl *N,N'*-diethylphosphoramidite (DDP) in the preparation of dibenzyl glycosyl phosphites.<sup>103</sup> Subsequent oxidation of the phosphite with hydrogen peroxide created the desired glycosyl phosphate.<sup>103</sup> Glycosyl phosphites and phosphates of a number of sugars were prepared in this manner, including 2,3,4,6-tetra-*O*-acetyl-D-glucose shown in Fig 2.9.<sup>103</sup>



**Fig 2.9 :** The use of DDP and 1,2,4-triazole in the synthesis of dibenzyl 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl phosphate.<sup>103</sup>

Treatment of 2,3,4,6-tetra-*O*-acetyl-D-glucose, **115**, with DDP in the presence of 1,2,4-triazole in anhydrous DCM under a nitrogen atmosphere resulted in the formation of dibenzyl 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl phosphite, **116**.<sup>103</sup> Oxidation of **116** with 30% hydrogen peroxide at  $-78^\circ\text{C}$  in THF afforded the desired dibenzyl 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl phosphate, **117**, in 98% yield.<sup>103</sup>

A slightly modified version of this approach was used in an attempt to phosphorylate alcohol **109**, whereby the previously used DDP was replaced with the more readily available compound *N,N'*-diisopropyl-di-*tert*-butyl phosphoramidite. Treatment of compound **109** with 1,2,4-triazole and *N,N'*-diisopropyl-di-*tert*-butyl phosphoramidite in anhydrous THF was followed by oxidation with a solution of mCPBA in anhydrous DCM, Fig 2.10.

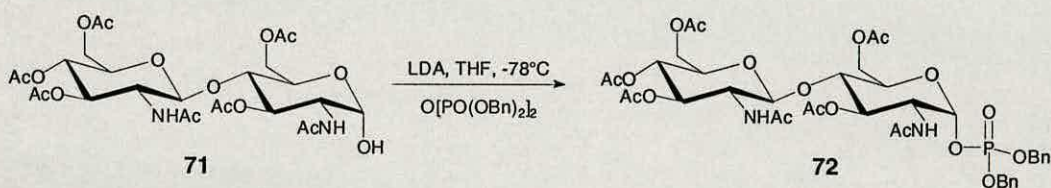


**Fig 2.10 :** Attempted phosphorylation of the alcohol using 1,2,4-triazole.



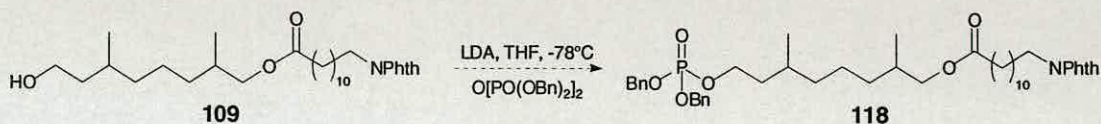
Unfortunately, phosphorylation of the alcohol was not accomplished. Analysis of the crude reaction mixture did not provide any evidence of the desired product and the compound obtained was not identified. It would appear that the lower acidity of 1,2,4-triazole ( $pK_a \sim 10$ , compared with tetrazole,  $pK_a \sim 5$ ) makes it an unsuitable replacement for tetrazole in the synthesis of a lipid-phosphite from *N,N'*-diisopropyl-di-*tert*-butyl phosphoramidite.

A method of phosphorylation that had been successfully used in the synthesis of dibenzyl chitobiosyl phosphate was then considered. This process involved treatment of chitobiose heptaacetate, **71**, with LDA at  $-78^\circ\text{C}$  in THF followed by reaction with tetrabenzyl pyrophosphate (TBPP) as shown in Fig 2.11.<sup>82</sup>



**Fig 2.11** : Phosphorylation of chitobiose heptaacetate using LDA and tetrabenzyl pyrophosphate.<sup>82</sup>

The main concern with this method was that the lipid chain would not withstand the reaction conditions. However, in the aforementioned reaction with chitobiose heptaacetate, the non-nucleophilic base, LDA, did not affect any of the acetate ester protecting groups so it was reasoned that the lipid should survive under such strongly basic conditions. A stirred solution of alcohol **109** in THF at  $-78^\circ\text{C}$  was duly treated with a 1.8M solution of LDA in 1:1:1 heptane/THF/ethylbenzene followed by tetrabenzyl pyrophosphate, Fig 2.12.

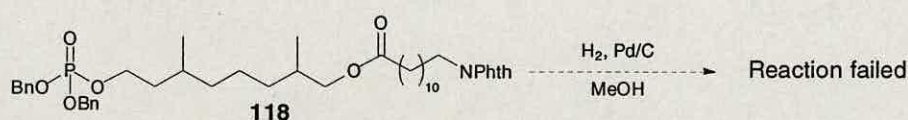


**Fig 2.12** : Attempted phosphorylation of the lipid using LDA and tetrabenzyl pyrophosphate.



The main compound recovered from the crude reaction mixture was thought to be the desired dibenzyl protected lipid phosphate, **118**. NMR analysis of this compound supported product formation. When compared to the  $^1\text{H}$  NMR of **109**, the  $^1\text{H}$  NMR for compound **118** showed additional peaks as follows: A multiplet integrating to ten protons at  $\delta_{\text{H}}$  7.46-7.37 ppm which was assigned to the protons belonging to the two  $\text{POCH}_2\text{Ph}$  groups, and a broad multiplet integrating to six protons observed at  $\delta_{\text{H}}$  5.21-5.13 ppm which was assigned to the  $\text{POCH}_2$  and two  $\text{POCH}_2\text{Ph}$  protons. The  $^{31}\text{P}$  NMR of compound **118** showed the presence of one phosphorous in the molecule with a singlet recorded at  $\delta_{\text{P}}$  -13.3 ppm. However, mass spectral analysis proved inconclusive with no evidence of a parent ion or any lipid fragment being formed. A further test was conducted to detect the presence of a lipid phosphate. This involved running a TLC of the compound and developing the plate with a molybdenum blue reagent. A blue spot should develop in the presence of a lipid phosphate but in this case the test proved to be negative. Although the NMR data obtained for compound **118** was consistent with the proposed structure, the lack of mass spectral and chromatographic data meant that there was no conclusive evidence for the formation of product **118** by this method.

More problems were encountered when attempting to deprotect the dibenzyl phosphate of **118**. Hydrogenation of lipid phosphate **118** over finely divided 10% palladium on charcoal as catalyst in anhydrous methanol gave a crude reaction mixture that proved difficult to purify, Fig 2.13.



**Fig 2.13** : Attempted deprotection of the dibenzyl phosphate.

Of the compounds obtained from this reaction no evidence was observed of any lipid phosphate by NMR, mass spectrometry or TLC analysis and although the compounds obtained could not be identified, they appeared to be fragments of the lipid chain. It would seem that the initial phosphorylation reaction with LDA and TBPP had been unsuccessful and that a mixture of lipid fragments and a phosphorus-



containing fragment co-eluted from the column during purification, giving what was thought to be the dibenzyl lipid phosphate.

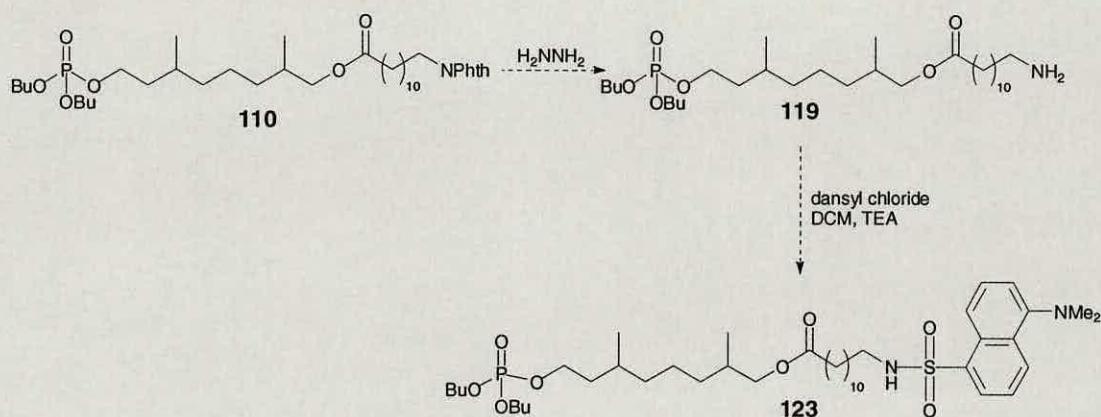
Fortunately, 1-*H*-tetrazole became available as a 3% solution in acetonitrile, making it possible to phosphorylate lipid **109**. The lipid phosphite was prepared from **109** by reaction with *N,N'*-diisopropyl-di-*tert*-butyl phosphoramidite and 1-*H*-tetrazole, Fig 2.5. Addition of a solution of mCPBA in DCM to the crude phosphite resulted in its successful oxidation to the desired di-*tert*-butyl lipid phosphate, **110**, in an overall yield of 77%.

Finally, it was possible to conduct the TFA mediated deprotection of the di-*tert*-butyl lipid phosphate generating the target novel dolichyl phosphate analogue, **99**, in 94% yield, Fig 2.5.

### 2.2.2. Synthesis of 3,7-dimethyl-8-[12-(4'-dimethylaminonaphthalene-1'-sulfonamido)dodecanoyloxy]octyl phosphate.

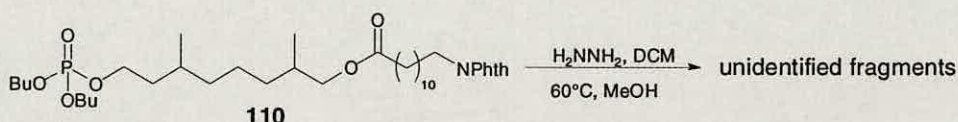
The first lipid analogue, **99** (Fig 2.5), synthesised as a replacement for the natural dolichol lipid was also to be used in the synthesis of a fluorescently labelled derivative. It was expected that the *N*-phthalimido group could be removed to expose a reactive primary amine. This, in turn, could be attached to solid support to further develop the enzyme-catalysed solid phase synthesis of *N*-glycans or derivatised to produce substrate mimics for specific glycosyltransferases.

Initially, a fluorescent label was to be attached to the amine for further study of the mannosyltransferase enzymes under investigation, Fig 2.14.



**Fig 2.14 :** Proposed synthetic route to the fluorescently labelled-lipid, **123**.

A literature search was conducted to source methods for the removal of the *N*-phthalimido group under conditions which would not prove detrimental to the remainder of the lipid chain, in particular the ester functional group. Several citations were identified which made use of hydrazine hydrate to convert the *N*-phthalamide to a primary amine in substrates where an ester was also present.<sup>104, 105</sup> In the first attempt, lipid **110** was dissolved in a mixture of anhydrous methanol and DCM and then treated with hydrazine hydrate. The reaction mixture was stirred for two hours at 60 °C in accordance with the experimental procedure of Mulzer and Brand.<sup>106</sup> TLC analysis of the crude reaction mixture after work up showed a number of products, separation of which proved to be difficult. Mass spectral analysis of the crude reaction failed to show any evidence of product. Two compounds were obtained upon purification but neither was identified as the desired product, Fig 2.15.



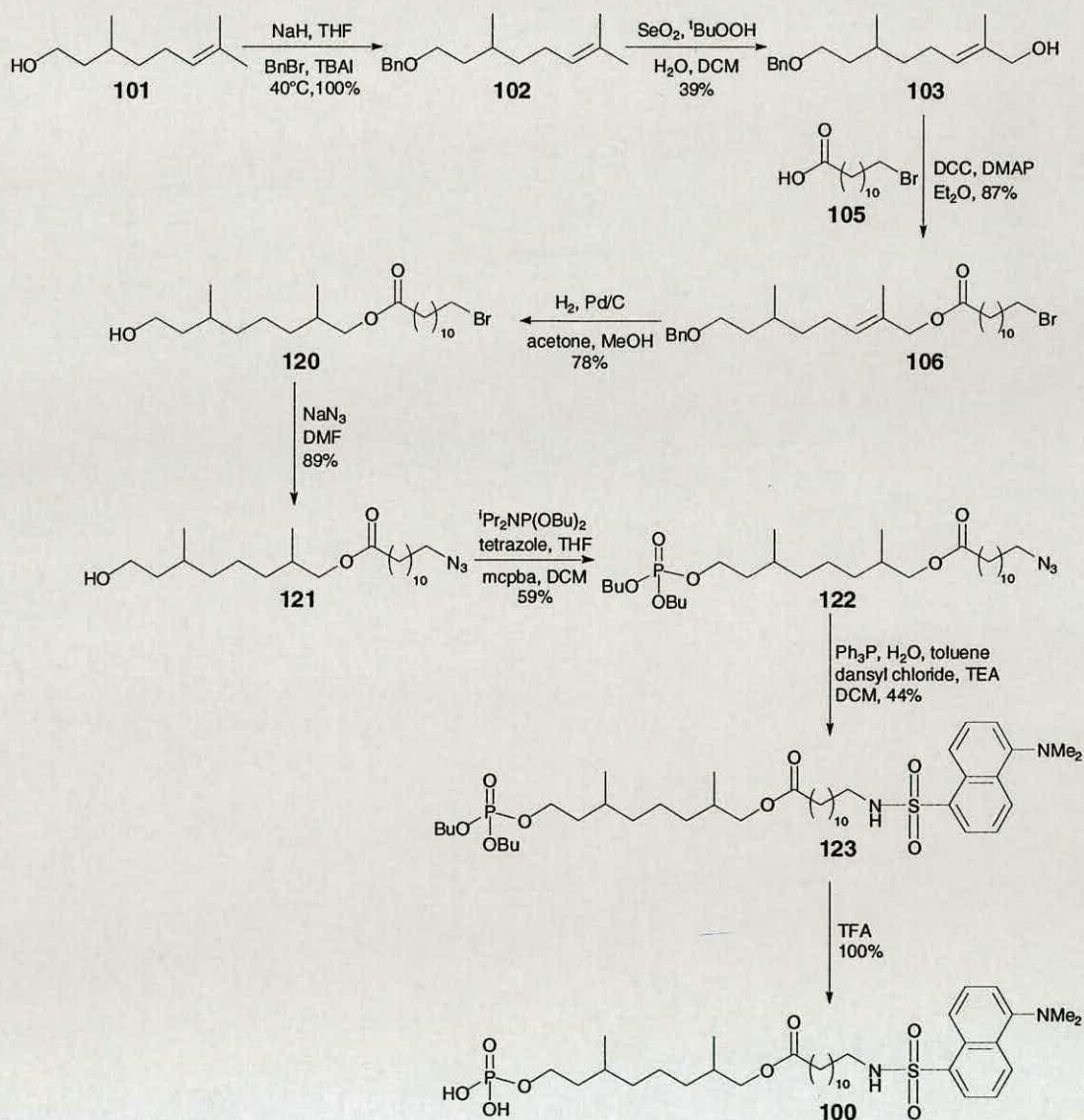
**Fig 2.15 :** Attempted hydrolysis of the *N*-phthalimido group using hydrazine hydrate.

Further reactions using hydrazine hydrate under different conditions such as varying the temperature, concentration of hydrazine and time of reaction all failed to produce the requisite amine, **119**, as shown in Fig 2.14. It appeared that hydrazine hydrate was incompatible with the ester moiety in the lipid chain since each reaction



designed to deprotect the *N*-phthalimido group appeared to cause disintegration of the whole lipid. In order to attach a fluorescent probe to this lipid, a new synthetic route had to be devised.

As can be seen from Fig 2.16 the new synthetic route began in the same way as in Fig 2.5 with benzyl protection of citronellol, **101**, followed by SeO<sub>2</sub>-catalysed oxidation to give the allylic alcohol, **103**. Esterification of the alcohol with 12-bromododecanoic acid in the presence of DMAP and DCC was conducted as before.



**Fig 2.16 :** Synthetic route to 3,7-dimethyl-8-[12-(4'-dimethylaminonaphthalene-1'-sulfonamido)dodecanoyloxy]octyl phosphate.

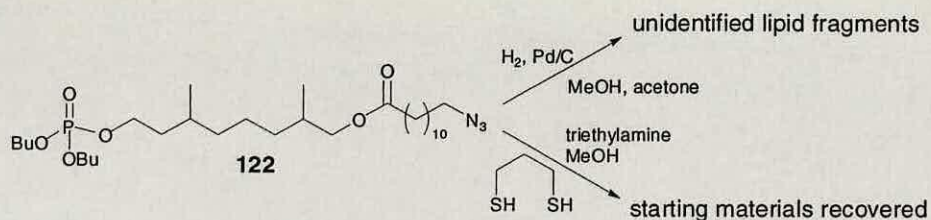
The new synthetic route deviates with the catalytic hydrogenation of lipid **106**. Removal of the benzyl protecting group and reduction of the double bond was carried out at an earlier stage than in the previous synthesis to facilitate the use of the azide. Deprotection of the alcohol and reduction of the double bond was achieved by treating the newly formed ester, **106**, with hydrogen gas in the presence of finely divided 10% palladium on charcoal to give lipid **120** in 78% yield, Fig 2.16.

The alkyl azide, **121**, was readily obtained from the alkyl bromide, **120**, by nucleophilic substitution of the bromide upon treatment with three equivalents of sodium azide in anhydrous DMF.<sup>107</sup> This efficient procedure afforded the alkyl azide, **121**, in 89% yield after purification. Since the reaction was carried out at room temperature and the product was isolated by column chromatography, it was possible to avoid the hazards associated with high temperature processes involving azides.

Phosphorylation of the deprotected alcohol was the next step to be undertaken. Again this was carried out using the method of Perich and Johns and involved treating the alcohol, **121**, with a solution of 1-*H*-tetrazole and *N,N'*-diisopropyl-di-*tert*-butyl phosphoramidite to give the phosphite.<sup>102</sup> Oxidation with mCPBA afforded the di-*tert*-butyl lipid phosphate, **122**, in 59% yield, Fig 2.16.

In order to obtain the amine required for attaching the fluorescent probe, it was necessary to find some way of reducing the azide. A variety of reagents have been reported in the literature for the reduction of azides and the most commonly used include borohydrides,<sup>108</sup> triphenylphosphine,<sup>109</sup> propane dithiol,<sup>110</sup> catalytic hydrogenation,<sup>111</sup> hydriodic acid<sup>112</sup> and FeCl<sub>3</sub> with sodium iodide (NaI).<sup>113</sup> Catalytic hydrogenation was the first process used to attempt the reduction of the azide as the lipid was thought to be stable under such conditions. A stirred solution of lipid, **122**, in a 2:1 mixture of acetone and methanol was treated with hydrogen gas over finely divided 10% palladium on charcoal, Fig 2.17.

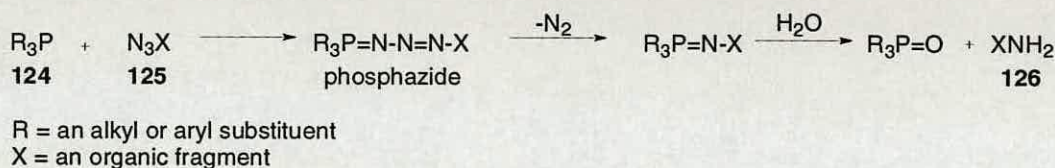




**Fig 2.17 :** Attempted reduction of the azide using (1) catalytic hydrogenation, and (2) propane-1,3-dithiol.

TLC analysis of the crude reaction mixture showed that all of the starting material had been consumed and that a number of products had formed. Separation of these compounds followed by NMR and mass spectral analysis did not show any indication of the desired amine but evidence for lipid fragmentation was observed.

The use of propane-1,3-dithiol as a selective reagent for the efficient reduction of alkyl and aryl azides to amines has been reported.<sup>110</sup> Propane-1,3-dithiol mediated reduction of azido substrates containing a wide variety of functional groups such as esters, amides, alkenes and alkynes were found to proceed selectively and in excellent yield.<sup>110</sup> However, when lipid **122** was dissolved in anhydrous methanol and treated with a three-fold molar excess of both propane-1,3-dithiol and triethylamine, a rapid and clean azide reduction was not observed. After stirring under nitrogen at room temperature for 2 days, only unreacted starting materials were isolated, Fig 2.17. This reaction was also quite difficult to handle given the stench from the thiol. It was decided that an efficient and more practical procedure would be sought. The Staudinger reaction occurs between an organic azide, **125**, and a trialkyl phosphite, **124**, to produce a phosphazo compound which is then hydrolysed to yield the primary amine, **126**, Fig 2.18.<sup>114</sup>



**Fig 2.18 :** General representation of the Staudinger reaction.<sup>114</sup>

A mixture of lipid **122** and triphenylphosphine in THF containing water was stirred under nitrogen at room temperature for 24 hours and then concentrated *in vacuo*, Fig 2.16. The crude reaction mixture was analysed by mass spectrometry and a clear product peak was observed. TLC of the crude substance indicated the presence of some side products. Great difficulty was experienced in trying to purify the amine and it was thought that the side products present would not interfere with attachment of the fluorescent probe. Consequently, the crude amine mixture was treated with one molar equivalent of both dansyl chloride and triethylamine, then stirred under an atmosphere of nitrogen for 16 hours, Fig 2.16. The lipid bearing a fluorescent probe, **123**, was isolated in 44% yield over this two-step reaction.

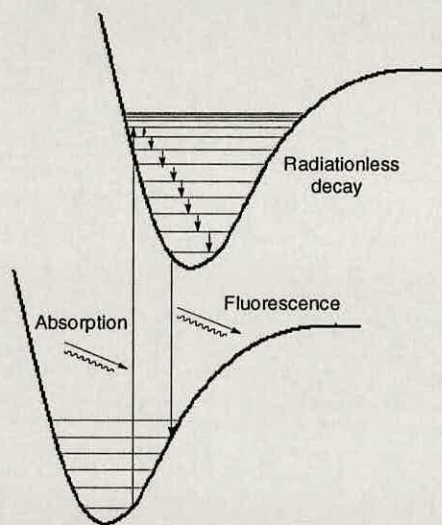
Dansyl chloride was chosen as the precursor to the fluorescent component of the target lipid because this non-fluorescent naphthalene derivative reacts with amines to form fluorescent dansyl amides that exhibit large Stoke shifts, along with environmentally sensitive fluorescent quantum yields and emission spectra.<sup>115</sup> Dansyl chloride has been used extensively to determine the *N*-terminal amino acid residue of proteins and to prepare fluorescent derivatives of drugs, amino acids, oligonucleotides and proteins for detection by numerous chromatographic methods.<sup>115</sup>

Once the fluorescent lipid **123** had been synthesised, the di-*tert*-butyl phosphate headgroup was deprotected in quantitative yield by treatment with TFA to afford the desired compound **100**, Fig 2.16.



### 2.3. Fluorescence spectra of the dansyl-amide lipid.

As previously mentioned, non-fluorescent dansyl chloride reacts with amines to form fluorescent dansyl amides that exhibit large Stoke shifts. The sequence of steps leading to fluorescence is illustrated in Fig 2.19.<sup>94</sup>

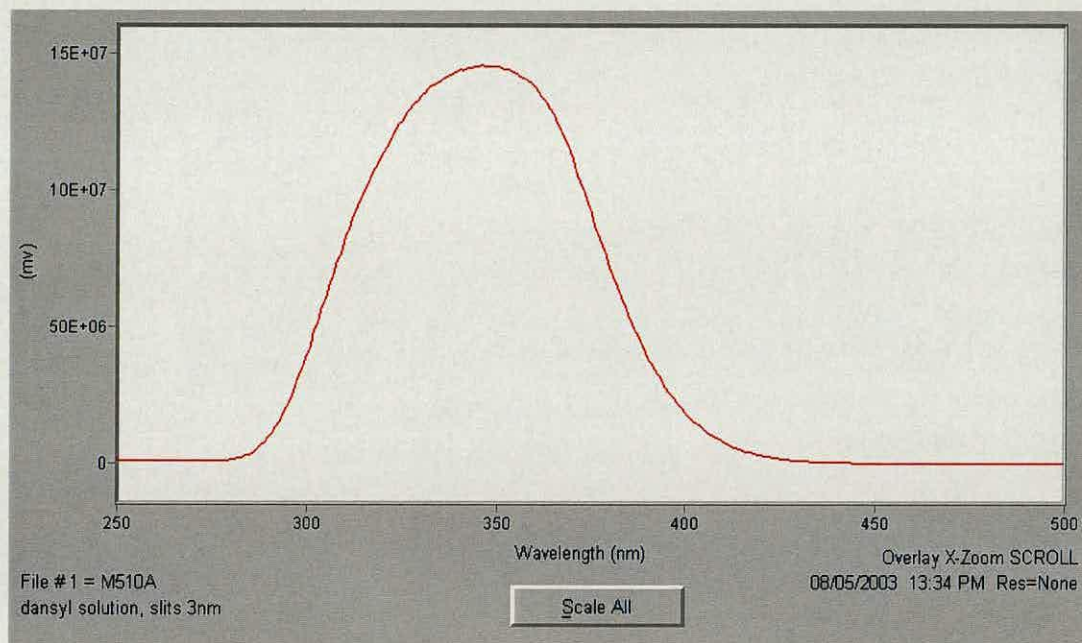


**Fig 2.19** : *Jablonski diagram<sup>94</sup> showing the sequence of steps leading to fluorescence.*

Absorption is a process whereby electromagnetic radiation interacts with matter to generate an electronically excited state, Fig 2.19.<sup>94</sup> The energy of this excited state may be lost in a variety of different ways. The most common is non-radiative decay, where the excess energy is transferred into the vibration and rotation of the surrounding molecules.<sup>94</sup> A radiative decay process is one in which a molecule discards its excitation energy as a photon of light.<sup>94</sup> Radiative decay occurs either in the form of fluorescence (where the spontaneously emitted radiation ceases immediately after the exciting radiation is extinguished) or phosphorescence (where the spontaneous emission may persist for long periods).<sup>94</sup>

For fluorescent studies involving the dansyl-sulfonamide substrate, **100**, the lipid was dissolved in anhydrous methanol and steady-state fluorescent measurements were carried out on a Spex Fluoromax spectrofluorometer.

The initial absorption took the molecule to an excited electronic state so that the absorption spectrum shown in Fig 2.20 was obtained with the emission monochromators set at 510nm.



**Fig 2.20 :** Absorption spectrum of the dansyl-amide lipid showing the excitation maximum at 347nm.

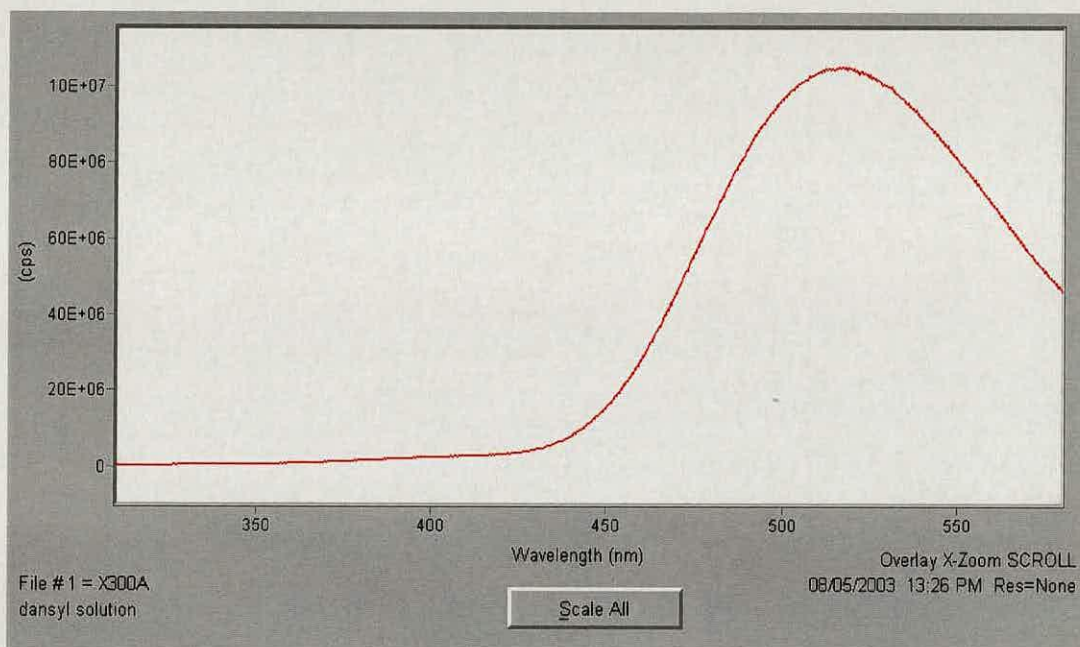
The strong band obtained is a characteristic uv-absorption peak for the incorporated dansyl chromophores, with its excitation maximum at 347nm.

In order for fluorescence to occur, the excited molecule is subjected to collisions with the surrounding molecules, and as it gives up energy it steps down the ladder of vibrational levels (non-radiative decay) to the lowest vibrational level of the excited electronic state, Fig 2.19.<sup>94</sup> If the surrounding molecules are unable to accept the larger energy difference needed to lower the molecule to the ground electronic state,



it may survive long enough to undergo spontaneous emission and emit the remaining excess energy as radiation giving a fluorescence spectrum, Fig 2.19.<sup>94</sup>

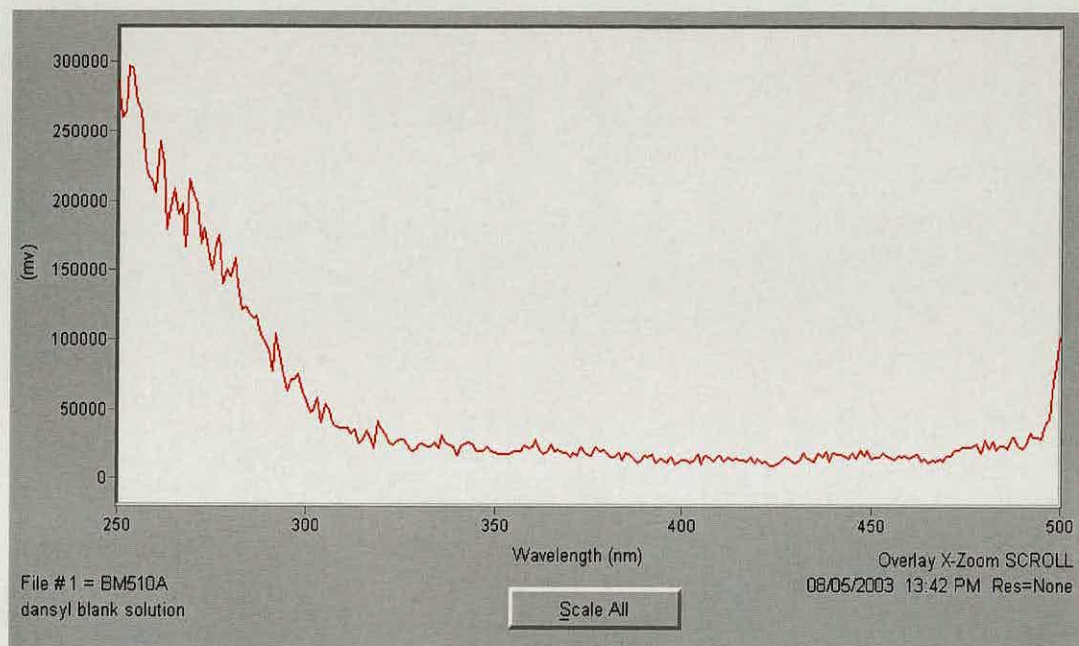
The fluorescence spectrum resulting from excitation of lipid **100** is illustrated in Fig 2.21.



**Fig 2.21 :** *Emission spectrum of the dansyl-amide lipid. The lipid is strongly fluorescent and has an emission maximum at 516nm.*

Excitation at 347nm resulted in a broad emission band with its peak at 516nm. In the polyatomic lipid under investigation so many vibrational levels exist that bands merged and a continuous broadband spectrum was recorded under low resolution, Fig 2.21.

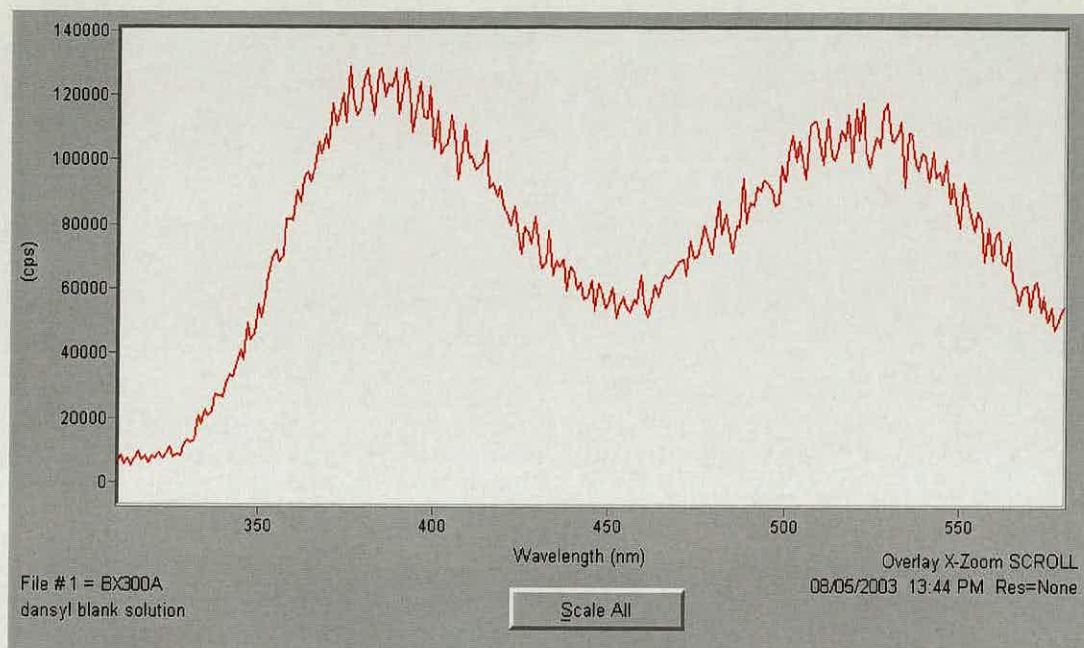
In order to ensure that dansyl chloride, the starting material, was non-fluorescent, this too was investigated. Excitation of a solution of dansyl chloride in anhydrous methanol with radiation of wavelength 510nm produced the absorption spectrum shown in Fig 2.22.



**Fig 2.22 :** *Absorption spectrum of dansyl chloride. The excitation maximum is below 300nm and does not interfere with the absorption (or overlap the area of absorption) of the dansyl-amide lipid.*

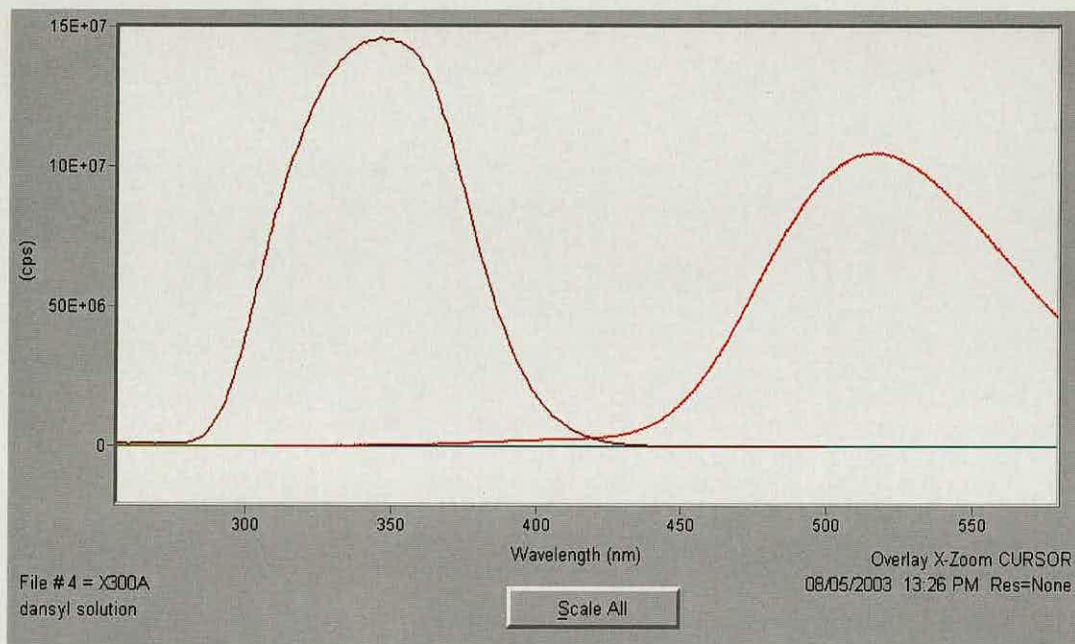
The excitation maximum occurred well below 300nm in this instance and, as can be seen from Fig 2.22, no absorption was observed in the region of 350nm, the wavelength corresponding to the strong absorption peak of the dansyl-sulfonamide lipid **100**. The emission spectrum obtained for dansyl chloride is illustrated in Fig 2.23.





**Fig 2.23 :** *Emission spectrum of dansyl chloride. The starting material is weakly fluorescent but this is insignificant compared to the strength of fluorescence observed with the dansyl-amide lipid.*

A very weak emission was detected with maxima at 385 and 524nm. The insignificance of this fluorescence is compounded by Fig 2.24 in which all four aforementioned spectra are overlaid.



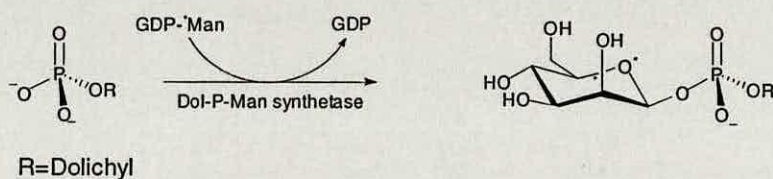
**Fig 2.24 :** All four spectra are shown together here, namely the absorption and emission spectra of dansyl chloride and the dansyl-amide lipid.

On the left hand side of this diagram are the excitation spectra of both the lipid **100** (large peak) and the dansyl chloride (flat line) and on the right hand side are the corresponding emission spectra. The excitation and emission spectra of dansyl chloride appear as a flat line close to zero when compared to the strength of absorption and emission observed for the fluorescently-labelled lipid. From this spectrum, it can be concluded that the strongly fluorescent lipid **100** had been successfully synthesised.



## 2.4. Biological testing of dolichyl lipid analogues.

The biological activity of the two lipid phosphates **99**, (Fig 2.5), and **100**, (Fig 2.16), were tested by assaying them with Dol-P-Man synthase for substrate specificity. As can be seen from Fig 2.25, Dol-P-Man synthase is the glycosyltransferase responsible for catalysing the stereoselective  $\beta$ -mannosylation of dolichyl phosphate to yield the mannosyl dolichyl phosphate diester.

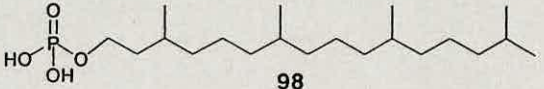
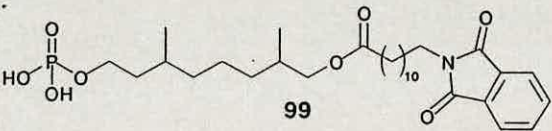
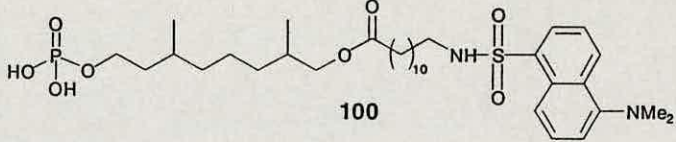


**Fig 2.25 :** *The transfer of mannose from GDP-Man to a lipid phosphate is catalysed by the enzyme Dol-P-Man synthase.*<sup>96</sup>

Since Dol-P-Man synthase is thought to possess a putatively conserved dolichyl binding site, it was reasoned that if a lipid phosphate was to act as a substrate for this enzyme, it may also act as a substrate for other mannosyltransferases involved in *N*-glycan biosynthesis.

The biological testing was carried out by Dr. Ines Sprung, School of Chemistry, University of Edinburgh. The lipid phosphates **99** and **100** (100.0  $\mu$ M) were incubated in parallel with phytanyl phosphate (100.0  $\mu$ M), **98** (Table 2), with radiolabelled GDP-[<sup>14</sup>C]-Man (0.1  $\mu$ Ci/mL) and crude microsomal fractions (1.0-2.0 mg) of *Saccharomyces cerevisiae* in a pH 7.5 buffer solution (1.0 mL) comprising 50.0 mM TrisHCl, 5.0 mM MgCl<sub>2</sub>, 10.0 mM mercaptoethanol and 0.5% Triton X-100 at 37°C for 1h. The aqueous phase was removed after centrifugation. The organic phase was washed twice with water and incorporation of radioactivity into organic soluble material was measured by scintillation counting. After this procedure, minimal counts were retained in the pellet. Evidence for the activity of the enzyme was provided by expressing it with the same cell strains that had previously been used within the research group.<sup>35 82 87 85</sup> A comparison of the results from these

tests with material that is known to be active confirmed that the enzyme used to attach radiolabelled mannose to lipids **98**, **99** and **100** was active. The results obtained are outlined in Table 2.

Lipid Phosphate	Incorporation relative to phytanyl phosphate
 <p style="text-align: center;"><b>98</b></p>	<b>1.00</b>
 <p style="text-align: center;"><b>99</b></p>	<b>0.77</b>
 <p style="text-align: center;"><b>100</b></p>	<b>0.14</b>

**Table 2.**

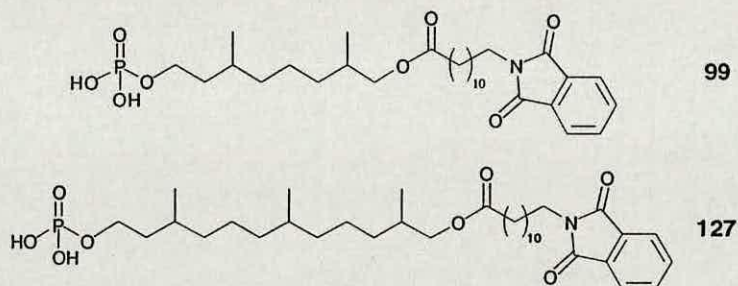
Evidence for the transfer of radiolabelled mannose from GDP-[<sup>14</sup>C]-Man to each lipid was provided by scintillation counting of an organic extract of the reaction. If the counts per minute of the organic extract were not above the background level (measured for the organic extract with no radioactive material) then no transfer of radiolabelled mannose to lipid had occurred.

As can be seen, the soluble acceptor lipid **99** caused a significant increase in the radiation levels of the extractable lipids. It compared well to phytanyl phosphate as a substrate, being 77% as active as phytanyl phosphate, **98**. Although the lipid bearing the fluorescent probe, **100**, only exhibited a relative activity of 14%, it was nevertheless still clearly recognised and mannosylated by the enzyme. It would appear, from these results, that there is less tolerance by the enzyme for the bulky sulfonamide group at the ω-position.



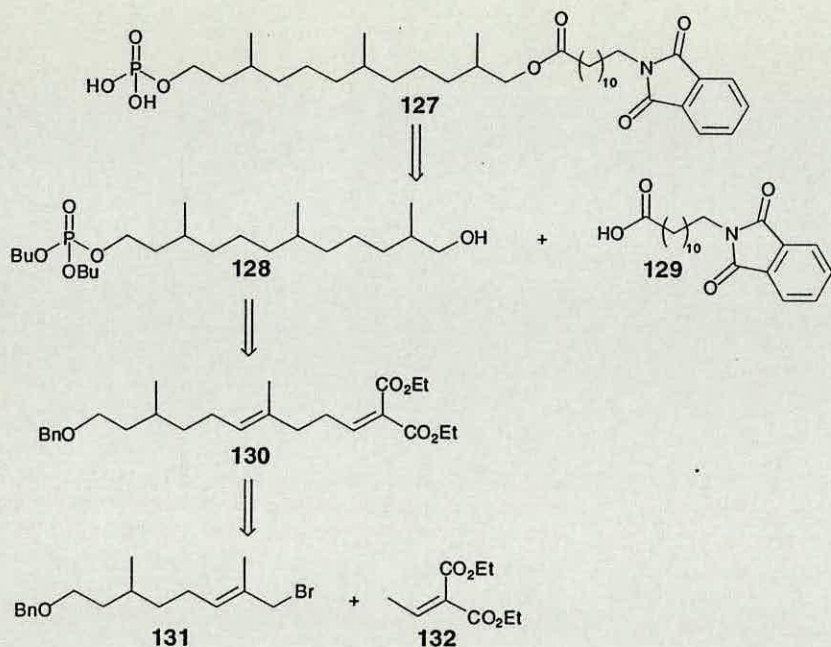
2.5. Attempted synthesis of 3,7,11-trimethyl-12-(12-*N*-phthalimidododecanoyloxy)dodecyl phosphate.

In order to obtain further insight into the nature of the dolichyl binding site of the glycosyltransferase enzymes involved in *N*-glycan biosynthesis, it was decided to investigate a structural variation of the novel dolichol analogue **99**. The new target lipid, **127**, is illustrated in Fig 2.26. This lipid contains an extra saturated isoprene unit. In this way it would be possible to study the effect that increased chain length has on enzyme activity.



**Fig 2.26 :** Novel target lipid extended in length by one isoprene unit.

To enhance the possibility that this substrate would be accepted by the glycosyltransferase enzymes under investigation, the new lipid **127** retained the structural features of lipid **99**. This lipid had showed 77% activity relative to phytanyl phosphate, **98**, in the biological assay. As can be seen in Fig 2.26, these structural features include the polar group at the  $\omega$ -position and the ester linkage in the lipid backbone, as well as the phthaloyl end unit. In order to devise a practical synthetic route to this lipid, a retrosynthetic analysis was carried out, Fig 2.27.



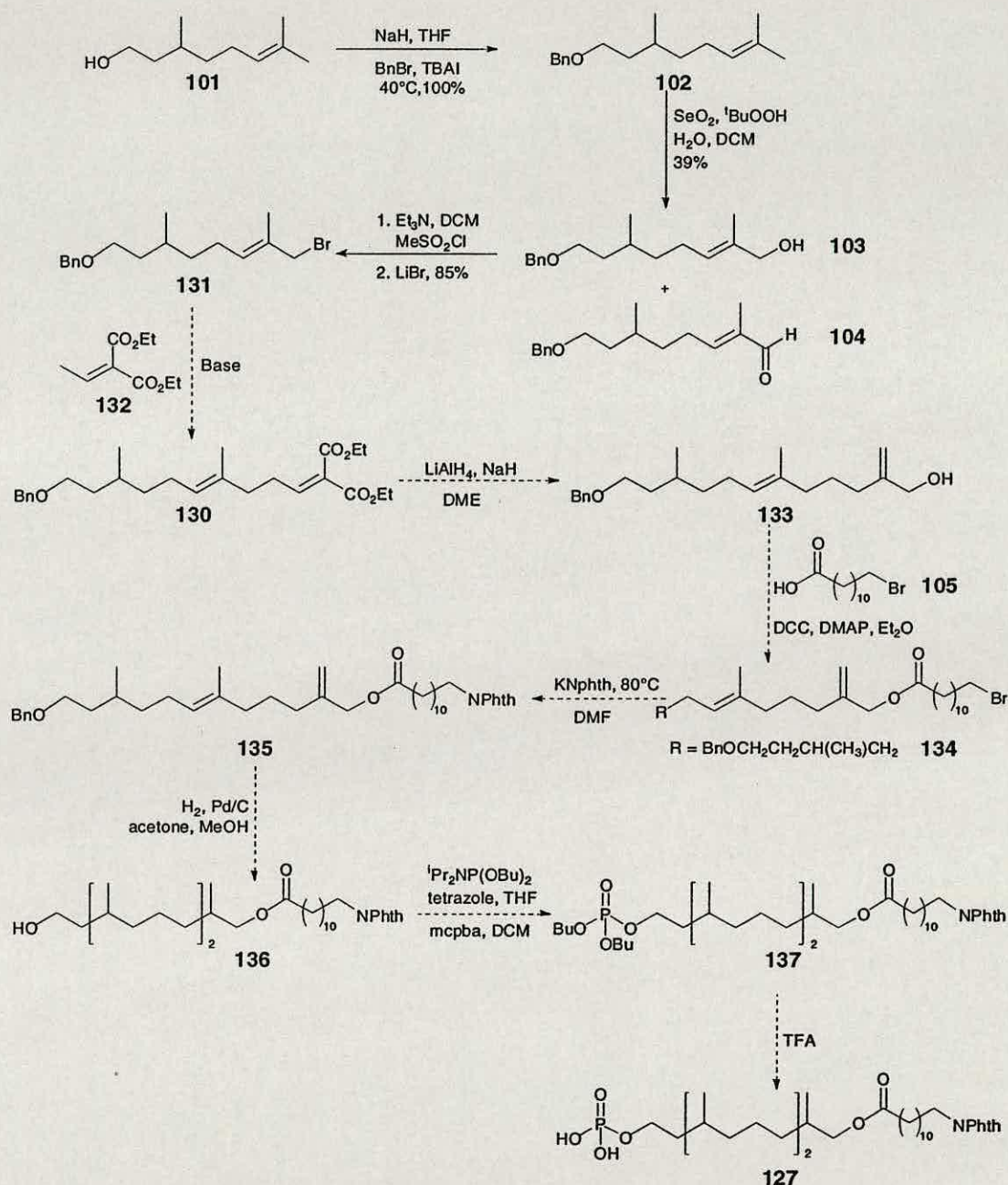
**Fig 2.27 : Retrosynthetic analysis of target lipid 45.**

Cleaving lipid **127** at the ester linkage generates synthetic equivalents **128** and **129**. Compound **129** is readily synthesised from commercially available 12-bromododecanoic acid, **105**, using chemical procedures applied in the synthesis of lipids **99** and **100**, Fig 2.5 and Fig 2.16. Literature precedent exists for the reduction of a diester into an allylic alcohol<sup>116</sup> and so it should be possible to obtain synthetic equivalent **128** from a substrate such as **130**. Disconnection of lipid **130** between carbons 8 and 9 would give rise to commercially available diethyl ethylidene malonate, **132**, and the easily synthesised citronellol derivative, **131**. On the basis of this information, a synthetic route to the desired lipid was devised as shown in Fig 2.28.

The synthesis of lipid **127** began with the benzyl protection of commercially available citronellol, **101**. As previously discussed, treating citronellol with sodium hydride in THF at room temperature for 1 hour, followed by heating to 40 °C in the presence of benzyl bromide and tetrabutylammonium iodide for a 4 hour period generated the benzyl protected alkene, **102**, in quantitative yield. Oxidation of the



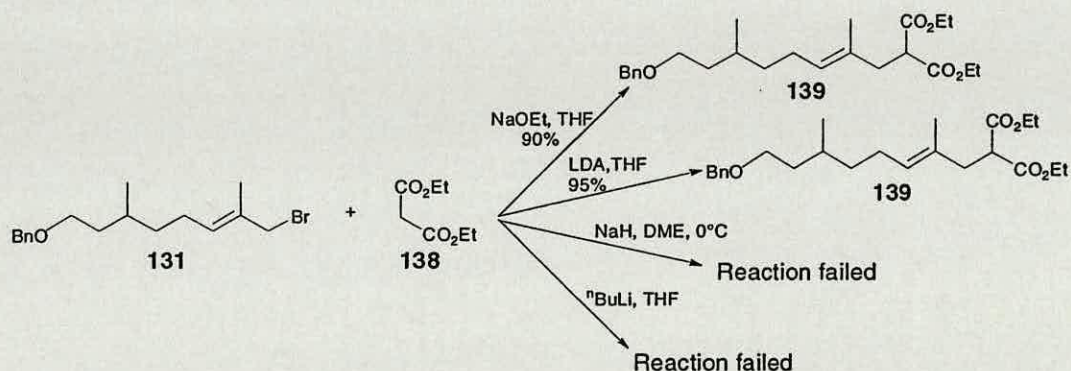
allylic end of **102** with catalytic selenium dioxide and aqueous *tert*-butyl hydroperoxide afforded the allylic alcohol, **103**, in 39% yield.



**Fig 2.28** : Proposed route for the synthesis of 3,7,11-trimethyl-12-(12-N-phthalimidododecanoyloxy)dodecanyl phosphate.

In order to prepare the citronellol derived lipid, **131**, for the attachment of diethyl ethyldenemalonate, **132**, the allylic alcohol had to be converted into a better leaving group. Conversion of the allylic alcohol into an allylic bromide was achieved in a two-step, one-pot process. A solution of the allylic alcohol, **103**, in anhydrous DCM was cooled to  $-50\text{ }^{\circ}\text{C}$  and treated with triethylamine. After stirring for 5 minutes under an atmosphere of nitrogen, a two-fold excess of methanesulfonyl chloride was added to the reaction mixture, which was then stirred at  $-50\text{ }^{\circ}\text{C}$  for 90 minutes.<sup>96</sup> Treating the methanesulfonate ester thus formed with a solution of lithium bromide in anhydrous THF resulted in nucleophilic displacement of the labile sulfonate ester by the bromide anion to form lipid **131** in 85% yield, Fig 2.28.<sup>96</sup>

Once the allylic bromide had been formed, it was anticipated that introduction of the new isoprene-like unit would be achieved by a simple nucleophilic displacement of the bromide by the diethyl ethyldene malonate anion. In order to find suitable conditions for such a process, test reactions were conducted using diethyl malonate, **138**, as shown in Fig 2.29.

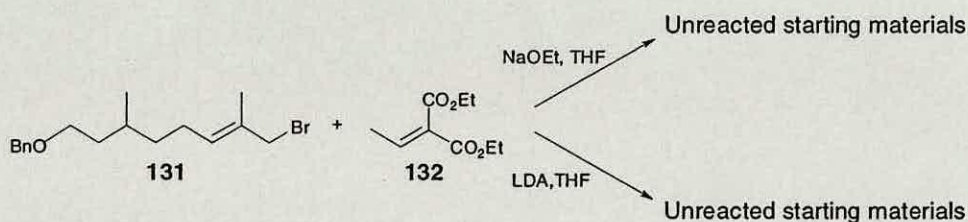


**Fig 2.29** : Test reactions to find a suitable reagent for the coupling of the allylic bromide with diethyl malonate.

The first procedure undertaken involved treating a solution of diethyl malonate, **138**, in anhydrous THF with one molar equivalent of sodium ethoxide to generate the anion.<sup>117</sup> Addition of the allylic bromide, **131**, to this solution resulted in the successful preparation of diethyl 2-(8-benzyloxy-2,6-dimethyloct-2-en-1-yl) malonate, **139**. However, when this method was applied to reaction with diethyl



ethylidene malonate, **132**, only unreacted starting materials were recovered from the reaction mixture, Fig 2.30.



**Fig 2.30 :** Attempted coupling of the allylic bromide with diethyl ethylidene malonate.

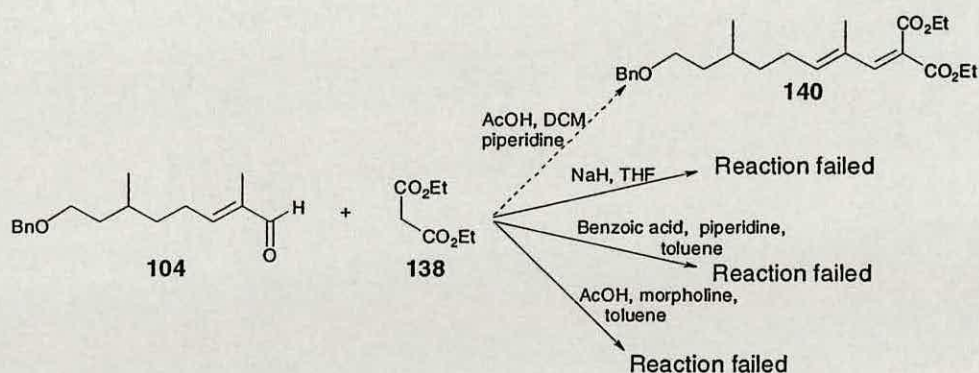
A second successful synthesis of diethyl 2-(8-benzyloxy-2,6-dimethyloct-2-en-1-yl) malonate, **139**, was performed by treating a solution of diethylmalonate, **138**, in anhydrous THF with one equivalent of 1.8 M solution of LDA in 1:1:1 heptane/THF/ethylbenzene and stirring at 0 °C under an atmosphere of nitrogen for 1 hour. Subsequent addition of a solution of the allylic bromide, **131**, in anhydrous THF afforded the desired lipid, **139**, in 95% yield, Fig 2.29. Unfortunately, when diethyl ethylidene malonate, **132**, was treated with LDA and the allylic bromide, **131**, under exactly the same conditions, none of the desired product was isolated. Some unreacted allylic bromide and another unidentified compound was recovered from the crude reaction mixture, Fig 2.30.

A further two test reactions were conducted using diethyl malonate as a model compound in order to find a suitable procedure for the synthesis of diethyl 12-benzyloxy-2-carbethoxy-6,10-dimethyldodec-2,6-dienoate, **130**. The first of these involved treating a solution of diethyl malonate, **138**, in anhydrous DME with 0.6 molar equivalents of sodium hydride and stirring the reaction mixture at 0 °C under an atmosphere of nitrogen before adding a solution of the allylic bromide, **131**, in anhydrous DME to the reaction mixture, Fig 2.29.<sup>118</sup> When this procedure failed to generate any product, a similar method was employed in which *n*-butyl lithium was used as a base. Again, a cooled solution of diethyl malonate, **138**, in anhydrous THF was treated with a 1.6 M solution of <sup>n</sup>BuLi in hexanes and stirred under an atmosphere of nitrogen at 0 °C for 40 minutes.<sup>119</sup> Addition of the allylic bromide,



**131**, failed once more to to give lipid **139**, Fig 2.29. Neither of these last two bases was used in reaction with diethyl ethylidene malonate.

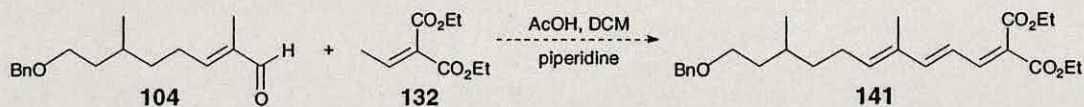
At this point a decision was taken to consider alternative routes to the desired substrate, **130**. One of the side products recovered from the selenium dioxide catalysed allylic oxidation of the benzyl protected alkene, **102**, is an  $\alpha,\beta$ -unsaturated aldehyde, **104**, Fig 2.28. Rather than converting this to the allylic alcohol in an inefficient and low-yielding reduction, it was thought that this aldehyde may be used in the synthesis of lipid **130**. Once again, several different test reactions were conducted using the readily available diethyl malonate as a model for the reaction, Fig 2.31.



**Fig 2.31** : Test reactions to find a suitable reagent for the coupling of the allylic aldehyde with diethylmalonate.

The first of these mixed carbonyl condensations was carried out in the presence of acetic acid and piperidine.<sup>120</sup> A stirring solution of the allylic aldehyde, **104**, in anhydrous DCM was treated with diethylmalonate, **138**, acetic acid and piperidine, then stirred under an atmosphere of nitrogen at room temperature for 18 hours. This reaction failed to create even a small amount of the desired product, diethyl 10-benzyloxy-2-carbethoxy-4,8-dimethyldec-2,4-dienoate, **140**, as illustrated in Fig 2.32. Even so a similar process was conducted using diethyl ethylidene malonate in place of diethyl malonate, Fig 2.32.

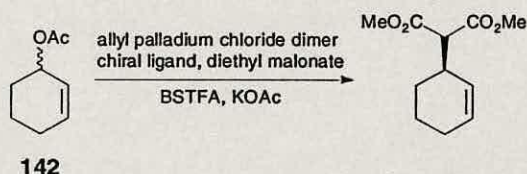




**Fig 2.32 :** Attempted coupling of the allylic aldehyde with diethyl ethylidenemalonate

Unfortunately, however, when the aldehyde, **104**, was treated with diethyl ethylidene malonate, **132**, in the presence of acetic acid and piperidine, a brown oil was recovered which could not be purified. A number of other aldol condensation procedures between the aldehyde and diethyl malonate were attempted as illustrated in Fig 2.32 but none of the reactions shown effected condensation of the two substrates.<sup>121, 122</sup> It was reasoned that if such reaction conditions were unsuitable for diethyl malonate, then they were also unlikely to bring about a successful coupling of the allylic aldehyde with diethyl ethylidene malonate.

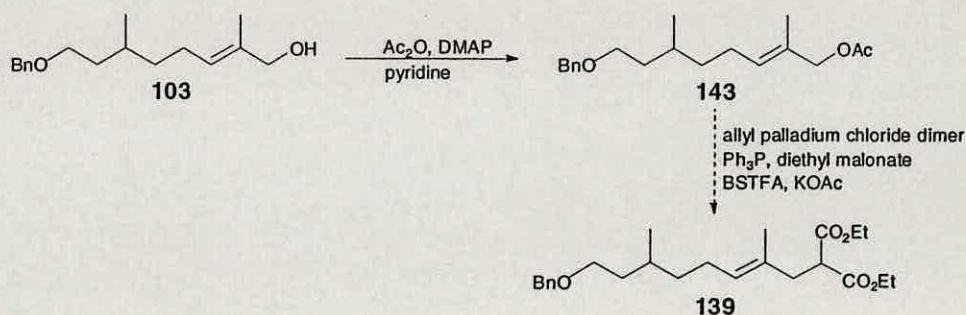
A final attempt to introduce the isoprene-like unit into the lipid chain involved a palladium-catalysed cross coupling reaction. The procedure used was based on a method developed by Ito *et al.* for the asymmetric allylic alkylation of both cyclic and acyclic alkenyl substrates, Fig 2.33.<sup>123</sup> Treatment of 2-cyclohexenyl acetate, **142**, with dimethyl malonate in the presence of a palladium complex in DCM using *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and potassium acetate as base, generated the desired diester in good yield (93%) and moderate enantiomeric excess (72% e.e.).<sup>123</sup>



**Fig 2.33 :** The asymmetric allylic alkylation of 2-cyclohexenyl acetate with diethyl malonate in a palladium-catalysed cross coupling reaction.<sup>123</sup>

A modified version of this method was used to couple diethyl malonate, **138**, and the protected citronellol derivative, **143**, as a model for the latter reaction with diethyl ethylidene malonate.

The allylic alcohol, **103**, was first acetylated in quantitative yield by treating it with acetic anhydride and pyridine in the presence of DMAP, Fig 2.34.



**Fig 2.34 :** Attempted alkylation of an acetate protected citronellol derivative with diethyl malonate using a palladium-catalysed cross coupling reaction.

Allylic alkylation of **143** commenced by stirring allyl palladium chloride dimer with triphenylphosphine in anhydrous DCM under an atmosphere of nitrogen for one hour at room temperature. A solution of the allylic acetate, **143**, in anhydrous DCM was added to the palladium based reaction mixture which was then cooled to  $-20\text{ }^\circ\text{C}$  and treated with diethyl malonate, BSTFA and catalytic potassium acetate. The reaction was maintained under an atmosphere of nitrogen at  $-20\text{ }^\circ\text{C}$  for 18 hours. However, after work-up, only unreacted starting materials were obtained. None of the desired product, **139**, was isolated from the reaction mixture. This procedure was not attempted using diethyl ethylidene malonate.

Unfortunately, the synthesis of the elongated lipid was not completed due to lack of time and the inability to find reaction conditions suitable for the synthesis of intermediate lipid, **130**.

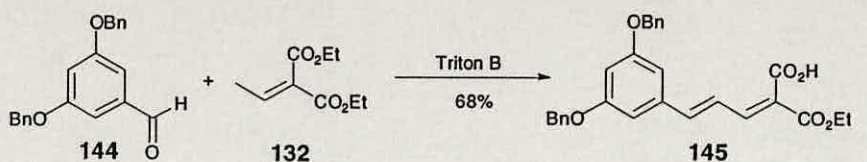


## 2.6. Conclusion.

Two novel analogues of the naturally occurring lipid, dolichyl phosphate, were synthesised. The first of these, lipid **99**, was found to exhibit 77% activity relative to phytanyl phosphate, **98**, in the biological assay with Dol-P-Man synthase. A new synthetic route had to be devised in order to attach a fluorescent probe to the  $\omega$ -end of lipid **99** so that the dansyl amide lipid, **100**, could be prepared. Although lipid **100** showed only 14% activity relative to phytanyl phosphate in the biological assay with Dol-P-Man synthase, it was nevertheless clearly recognised and mannosylated by the enzyme. In addition, a fluorescence study of lipid **100** showed that this substrate is indeed strongly fluorescent. The environmentally sensitive fluorescent quantum yields and emission spectra that a dansyl amide provides will be useful for further study of the unknown mannosyltransferases active in the early pathway of *N*-glycan biosynthesis. Finally, work towards the synthesis of an analogue of lipid **99**, namely lipid **127**, which incorporates an extra isoprene unit, was conducted.

## 2.7. Future Work.

Since this work was discontinued, literature precedent has been found for the condensation of diethyl ethylidene malonate, **132**, with 3,5-dibenzoyloxybenzaldehyde, **144**, in the presence of methanolic benzyltrimethylammonium hydroxide (Triton B) Fig 2.35.<sup>124</sup>



**Fig 2.35 :** *The condensation of diethyl ethylidenemalonate with 3,5-dibenzoyloxy benzaldehyde.*<sup>124</sup>

The half methyl ester, **145**, was recovered in 68% yield. In future, this procedure may lead to the successful incorporation of diethyl ethylidene malonate into the lipid chain thereby giving the ability to create longer multifunctionalised lipids.

In the event that suitable conditions cannot be found to attach diethyl ethylidene malonate to the allylic system, further work could be conducted using the lipid homologue diethyl 2-(8-benzoyloxy-2,6-dimethyloct-2-en-1-yl) malonate, **139**.

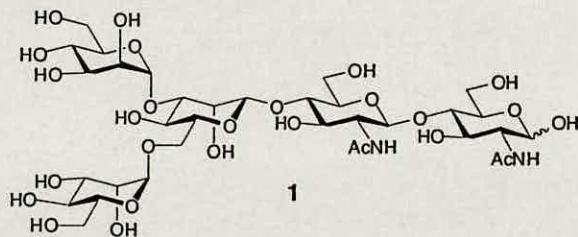


### 3. Synthesis of lipid-linked carbohydrates and *N*-linked chitobiose.

#### 3.1. Introduction.

As previously discussed in section 1.1, the oligosaccharide side-chains of glycoproteins are ubiquitous in nature and can play many roles in biological activity of the parent protein.<sup>3</sup> Glycoproteins are fundamental to many important biological processes including fertilisation, immune defence, viral replication, cell growth, cell-cell adhesion, degradation of blood clots and inflammation.<sup>4</sup> The glycoproteins found on cell-surface membranes play a role in intercellular recognition, acting as receptors for hormones, proteins and viruses.<sup>8</sup> They are also able to regulate the transportation of proteins between cells and as such act as signal substances in cell metabolism.<sup>64</sup>

The biological activity of glycoproteins is largely due to the terminal sequences of the oligosaccharide portion, in other words the outer chains or antennae.<sup>64</sup> In the case of *N*-linked glycoproteins, these outer chain oligosaccharides are attached to the protein residue via a pentasaccharide core, **1**, comprised of two *N*-acetylglucosamine residues and three mannose units, Fig 3.1.

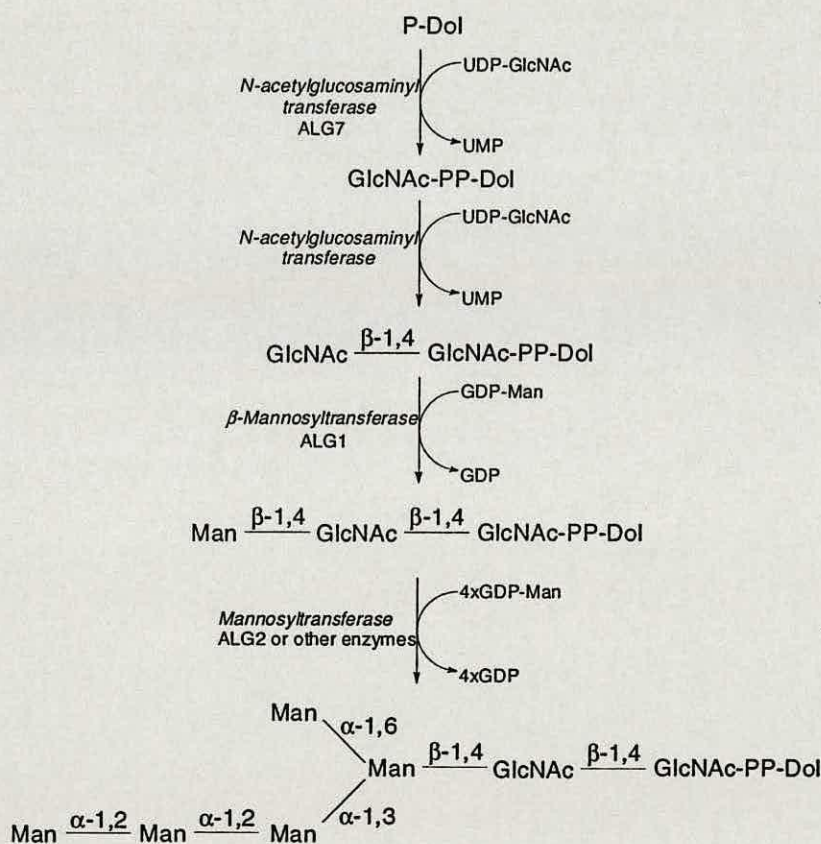


**Fig 3.1 :** *The pentasaccharide core common to all N-glycan species.*

In view of the biological importance of the oligosaccharide portion of glycoproteins, it is imperative that such species can be readily synthesised since biogenic material is frequently difficult to obtain. Modified antennae could provide information on the nature of the sites of attachment and thus on the mechanism of the biological interaction between the oligosaccharide side-chains and surrounding entities.<sup>64</sup>

The synthesis of oligosaccharides has already been discussed in some detail (sections 1.3 and 1.4) and from this it is evident that producing complex oligosaccharides is not always a simple task. Much research<sup>125</sup> has been conducted to find suitable chemical reactions and combinations of chemical and enzymatic techniques in order to establish facile methods of preparing oligosaccharides. Our work has focussed on the application of glycosyltransferase enzymes in the synthesis of *N*-linked glycoproteins.<sup>35, 82, 85</sup>

Many of the enzymes involved in the biosynthesis of *N*-linked glycoproteins have already been isolated and characterised. However, some of those enzymes involved in the early stages of *N*-glycan biosynthesis remain unidentified. The initial steps in the biosynthesis of *N*-linked glycoproteins are illustrated in Fig 3.2.



**Fig 3.2 :** Steps to the synthesis of the dolichyl pyrophosphate-linked heptasaccharide,  $\text{Man}_5\text{GlcNAc}_2\text{-PP-Dol}$ . The linkage of each individual glycosyl residue and the known loci coding for the corresponding glycosyltransferases are indicated.

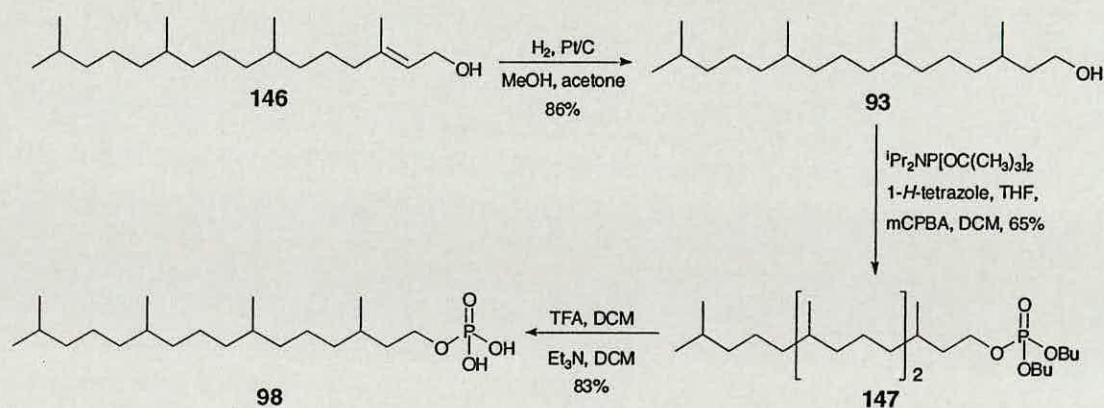


As can be seen from Fig 3.2, the enzymes responsible for the addition of the last four mannose residues are not known. Studies have found that ALG2 mutant cells accumulate  $\text{Man}_2\text{GlcNAc}_2\text{-PP-Dol}$  suggesting that the alg2 protein is a mannosyltransferase active in the early pathway of oligosaccharide assembly.<sup>36</sup> However, no specific enzymatic activity for this protein has been demonstrated and additional mannosyltransferases required for the assembly of  $\text{Man}_5\text{GlcNAc}_2\text{-PP-Dol}$  remain to be identified.

Work within our group has been aimed at establishing the structure and function of alg2; in particular whether it plays a role in attaching one or more of the last four mannose units of the dolichyl pyrophosphate-linked heptasaccharide shown in Fig 3.2.

### 3.2. Synthesis of phytanyl-linked chitobiose.

In order to investigate the role of alg2 it is necessary to have readily available supplies of the lipid-linked trisaccharide acceptor substrate, Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-PP-Dol. It has previously been shown that the much shorter lipid, phytanol, can be successfully used as a substitute for the natural dolichol lipid.<sup>35</sup> Since phytanyl phosphate, **98**, is readily available in gram quantities from phytol, **146**, by catalytic hydrogenation, it was decided to prepare a phytanyl-linked trisaccharide acceptor substrate. The synthesis of this substrate was achieved using a combination of chemical and enzymatic techniques and began with the preparation of the lipophilic membrane anchor, phytanyl phosphate, **98**, as illustrated in Fig 3.3.

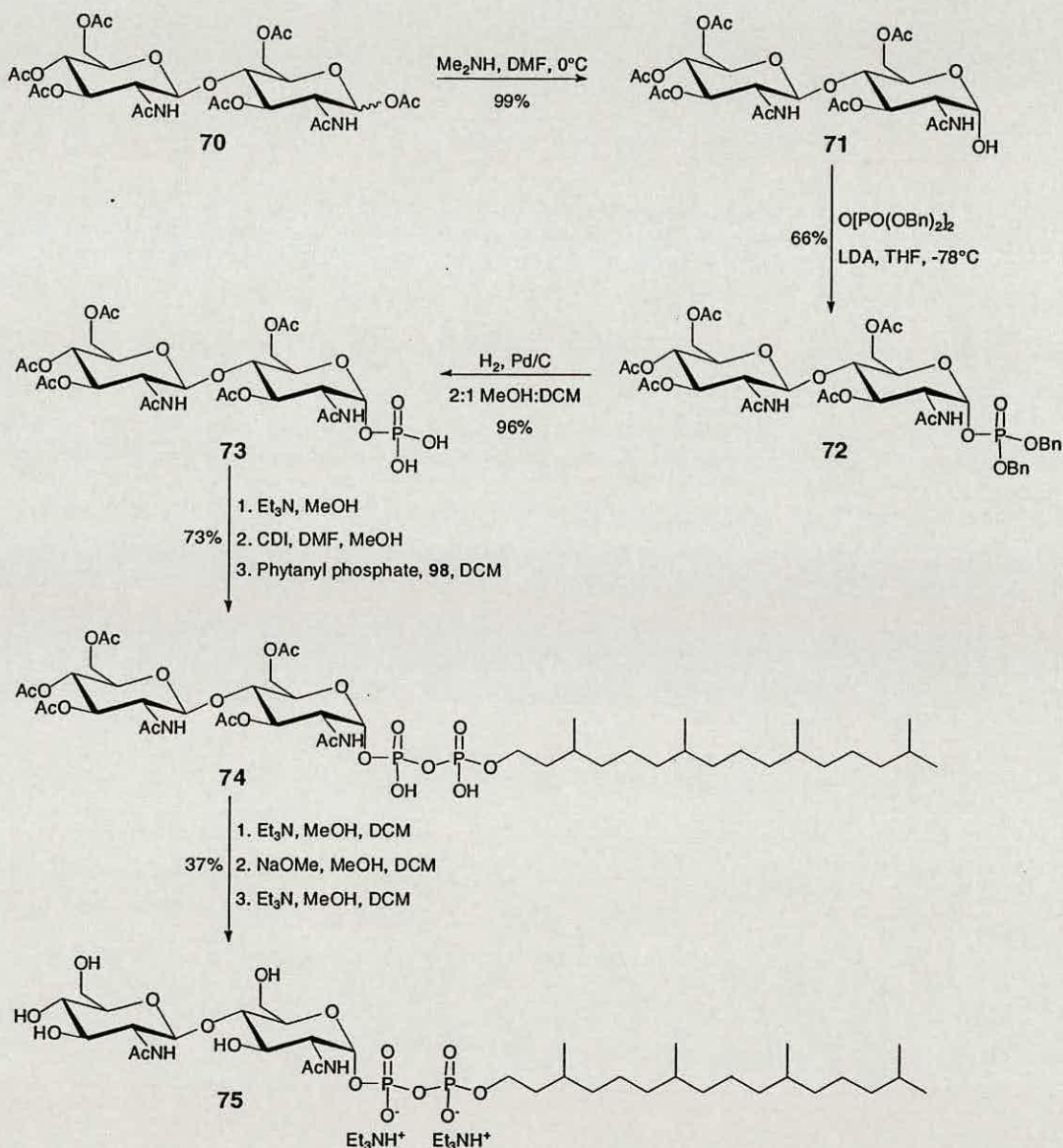


**Fig 3.3 :** The synthesis of phytanyl phosphate from commercially available phytol.<sup>35</sup>

The synthesis of phytanyl phosphate, **98**, began with the catalytic hydrogenation of commercially available phytol, **146**.<sup>35</sup> A solution of phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol), **146**, in anhydrous methanol was treated with hydrogen gas over finely divided 5% platinum on charcoal.<sup>35</sup> The reduction of the double bond proceeded efficiently, generating the saturated lipid, **93**, in 86% yield. Phosphorylation of the saturated alcohol, **93**, was achieved using conditions developed by Perich and Johns, where treatment of the alcohol with *N,N'*-di-*tert*-butyl diisopropylphosphoramidite and 1-*H*-tetrazole resulted in the formation of a phosphite.<sup>102</sup> The phosphite was not isolated and immediate oxidation of the crude reaction mixture with a solution of mCPBA in anhydrous DCM resulted in 65% yield of the desired lipid, di-*tert*-butyl phytanyl phosphite, **147**. Finally, TFA mediated



deprotection of the di-*tert*-butyl lipid phosphate **147** was conducted to generate phytanyl phosphate, **98**, in 83% yield. This deprotected lipid phosphate was to be coupled to chitobiose phosphate, **73**, to create a lipid-linked disaccharide. The synthesis of the sugar-phosphate, as well as the coupling reaction, is illustrated in Fig 3.4.



**Fig 3.4 : Synthesis of phytanyl-linked chitobiose.**<sup>82</sup>

The selective anomeric deacetylation of commercially available chitobiose octaacetate, **70**, was the first step in the synthetic route to the lipid-linked disaccharide, **75**.<sup>82</sup> This was achieved by treating a solution of chitobiose octaacetate,

**70**, in anhydrous DMF with a 2M solution of dimethylamine in DMF.<sup>82</sup> The resulting hemiacetal disaccharide **71** was obtained in quantitative yield, a marked improvement on previously reported syntheses of this compound, where selective 1-*O*-deacetylation of chitobiose octaacetate had been achieved in only 82% yield when using hydrazine acetate.<sup>35</sup>

The chitobiose heptaacetate **71** was then treated with a 1.8 M solution of lithium diisopropylamide (LDA) in 1:1:1 heptane/THF/ethylbenzene at  $-78^{\circ}\text{C}$  with the subsequent *in situ* addition of tetrabenzyl pyrophosphate.<sup>82</sup> This reaction afforded selectively the  $\alpha$ -glycosyl phosphate dibenzyl ester, **72**, in 66% yield, Fig 3.4. Hydrogenolysis of the benzyl groups was achieved in the presence of 10% palladium on charcoal under an atmosphere of hydrogen gas to give the heptaacetyl chitobiosyl phosphate, **73**, in 96% yield.<sup>82</sup>

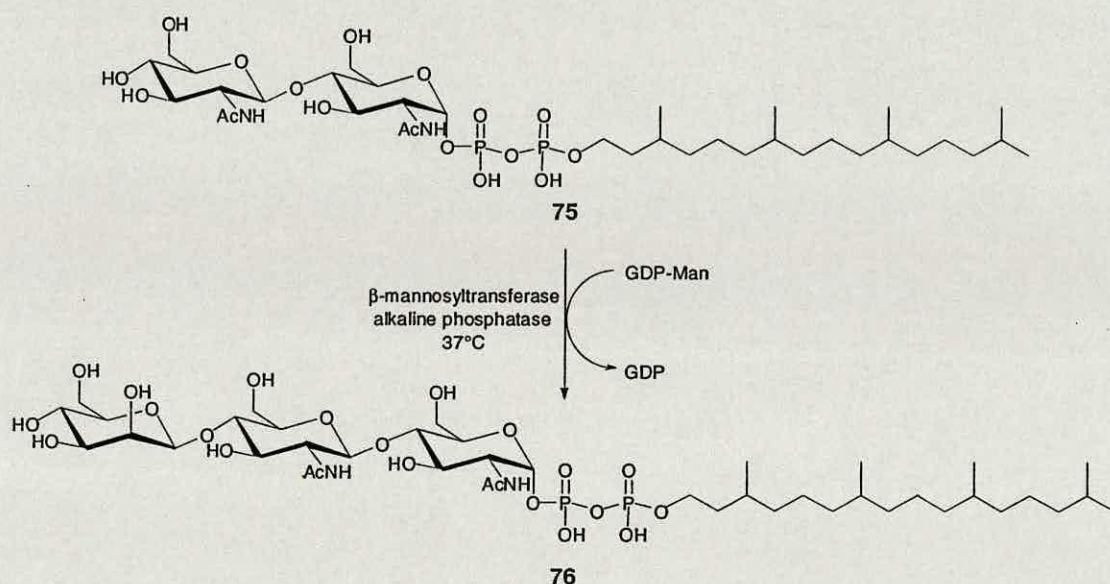
Conversion of phosphate **73** to its triethylammonium salt was followed by treatment with *N,N'*-carbonyldiimidazole in DMF to form an intermediate imidazole-carbonyl phosphate anhydride.<sup>82</sup> The addition of methanol to quench any excess *N,N'*-carbonyldiimidazole was followed by the addition of phytanyl phosphate to afford the phytanyl pyrophosphoryl chitobioside, **74**, in 73% yield.<sup>82</sup> Saponification of the protected pyrophosphate derivative, **74**, using sodium methoxide gave the acceptor substrate, **75**, in 37% yield.<sup>82</sup>

In order to further confirm the identity of **74** and **75** the analytical data for these compounds was compared to that published in the literature.<sup>82</sup> When this synthesis was first carried out the identity of the lipid-linked trisaccharide was confirmed by cleaving the trisaccharide from phytanyl pyrophosphate and comparing the NMR of the  $\text{Man}\beta 1\text{-4GlcNAc}\beta 1\text{-4GlcNAc}$  thus generated, to that of a sample of  $\beta$ -mannosyl-linked trisaccharide isolated from natural sources.<sup>82</sup>



### 3.3. Synthesis of the phytanyl-linked trisaccharide, Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc.

The preparation of the immobilised  $\beta$ -mannosyltransferase enzyme needed for the synthesis of the lipid-linked trisaccharide was conducted by Dr Ines Sprung, School of Chemistry, University of Edinburgh. A culture of *E. coli* cells harbouring the plasmid pLR36 yielded  $\beta$ -mannosyltransferase activity in cleared-cell lysates. Purification and immobilisation of the  $\beta$ -mannosyltransferase was achieved by passing the cleared-cell lysate through a nickel (II) charged affinity column followed by washing to remove any impurities.<sup>82</sup> The resulting functionally pure biocatalyst was used in the synthesis of the lipid-linked trisaccharide, **76**, as shown in Fig 3.5.<sup>82</sup>



**Fig 3.5 :** The formation of a phytanyl-linked trisaccharide using an immobilised  $\beta$ -mannosyltransferase.<sup>82</sup>

Pellets of recombinant *E. Coli* were stored at  $-80^{\circ}\text{C}$ . After rapid thawing to promote lysis, and the addition of DNaseI and Triton X-100, the cleared-cell lysate (25.0 mL) was applied to a column containing His-Bind resin (10.0 mL).<sup>82</sup> To ensure maximum immobilisation of the  $\beta$ -mannosyltransferase, the cleared-cell lysate was passed through the column twice and subsequently washed with binding buffer (100.0 mL)

and mannosyltransferase buffer (30.0 mL).<sup>82</sup> Evidence for the activity of the enzyme was provided by expressing it with the same cell strains that had previously been used within the research group.<sup>82 87 85</sup> A comparison of the results from these tests with material that is known to be active confirmed that the enzyme used in the conversion of **75** to **76** was active. The mannosyltransferase activity was assayed at 37°C by incubation of 50% (v/v) enzyme-charged resin in mannosyltransferase buffer (20.0 mL) with final concentrations of 160.0 µM **75** and 200.0 µM GDP-[2-<sup>3</sup>H]mannose (0.8 µCi/mL).<sup>82</sup> The reaction was shaken at 100 rpm at 37°C for 30 min followed by the addition of alkaline phosphatase (8 U); shaking was continued for a further 1h. The phytanyl-linked trisaccharide, **76**, was separated from the resin by vacuum filtration. Dr Sprung also completed this enzyme catalysed reaction.

Evidence for the formation of the lipid-linked trisaccharide, **76**, was provided by cleaving a sample of the trisaccharide from phytanyl pyrophosphate with aqueous HCl. A comparison of the NMR of the Manβ1-4GlcNAcβ1-4GlcNAc thus generated, to that of a sample of β-mannosyl-linked trisaccharide isolated from natural sources confirmed that the lipid-linked trisaccharide, **76**, had been formed.<sup>82</sup>

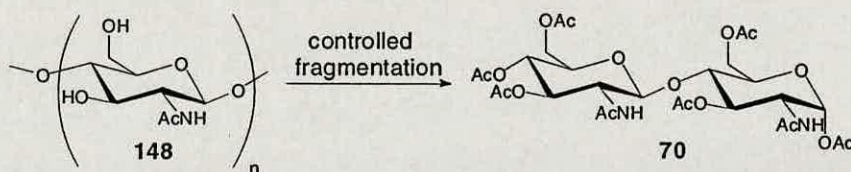
The lipid-linked trisaccharide is to be used to investigate the nature of ALG2 and any other enzymes involved in the addition of four mannose residues to form the lipid-linked heptasaccharide as illustrated in Fig 3.2.



### 3.4. Synthesis of phytanyl-linked glucosamine.

Although the synthesis of the phytanyl pyrophosphoryl chitobioside **75** outlined above is generally quite efficient, it has a major drawback in the high cost of the chitobiose octaacetate, **70**, starting material. A price of £200 per gram of starting material inhibits the large-scale production of the lipid-linked disaccharide needed to synthesise the lipid-linked trisaccharide for studies involving ALG2. Chemical syntheses of disaccharide **70** have been reported.<sup>126, 127</sup> However, they suffer from problems of selective protection and stereo- and regioselectivity discussed previously in relation to the chemical synthesis of oligosaccharides.

Alternatively, chitobiose octaacetate, **70**, can be obtained by controlled fragmentation and acetylation of chitin, **148**, Fig 3.6.<sup>38</sup>

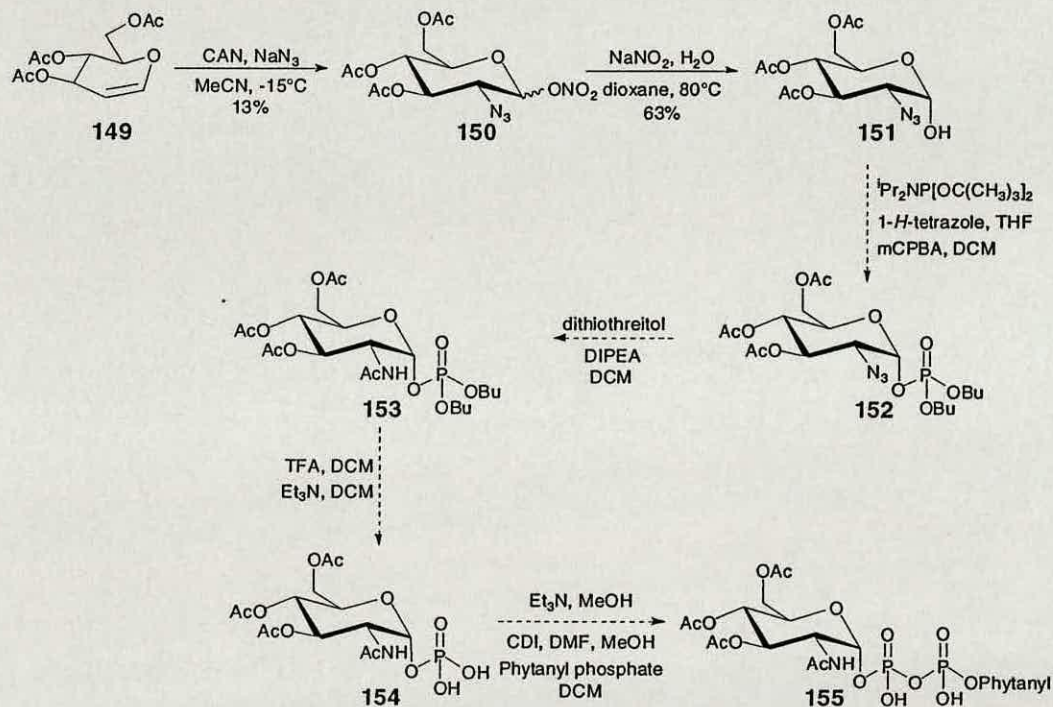


**Fig 3.6 :** *The synthesis of chitobiose octaacetate by the controlled fragmentation of chitin.*<sup>38</sup>

Several methods have been reported for the controlled fragmentation of chitin, **148**, including fluorohydrolysis,<sup>128</sup> enzymatic degradation<sup>129</sup> and acidic hydrolysis.<sup>38</sup> Although the use of hydrogen fluoride and the enzymatic method offer reasonably good yields of disaccharide **70**, neither technique is suitable for large-scale preparation work. Controlled acidic hydrolysis of chitin has also been found to be a complex, unreliable and low-yielding procedure.<sup>38</sup>

In view of the difficulties associated with obtaining chitobiose octaacetate, we decided to devise a cheaper, more robust synthetic route to the lipid-linked disaccharide, which would involve a combination of chemical and enzymatic methodologies. It was hoped that a chemically synthesised phytanyl-linked

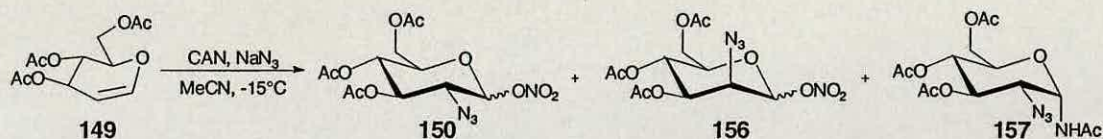
monosaccharide could be modified by an enzyme-catalysed reaction, thereby giving the desired phytanyl pyrophosphoryl chitobioside. The proposed synthetic route to the lipid-linked monosaccharide is outlined in Fig 3.7.



**Fig 3.7 :** Proposed synthetic route to phytanyl-linked glucosamine using tri-O-acetyl-D-glucal as starting material.

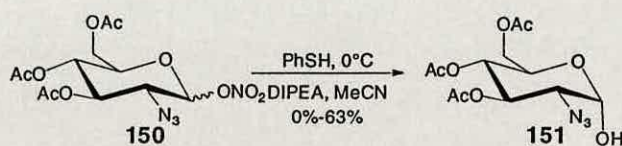
The synthesis of the lipid-linked monosaccharide began with the azidonitration of 3,4,6-tri-O-acetyl-D-glucal, **149**, a relatively cheap starting material costing £33 per 25 grams. Reaction of 3,4,6-tri-O-acetyl-D-glucal, **149**, with excess ceric ammonium nitrate (CAN) and sodium azide in anhydrous acetonitrile under an atmosphere of nitrogen at  $-15^\circ\text{C}$  was conducted according to the procedure developed by Lemieux and Radcliffe.<sup>130</sup> It was necessary to use an excess of both CAN and sodium azide since some of these reagents are lost in the ceric oxidation of azide to nitrogen gas.<sup>130</sup> The desired 2-azido-1-nitrate addition product, **150**, was isolated in a disappointing yield of 13%. The low yield of product recovered from this reaction may be attributed to the formation of a number of side-products that were difficult to separate, Fig 3.8.<sup>130</sup>





**Fig 3.8 :** Azidonitration of tri-O-acetyl-D-glucal and some of the by-products of the process.

Denitration of the azido-nitrate sugar, **150**, was the next step to be attempted. This process was first carried out by treating a cooled solution of 2-azido-3,4,6-tri-O-acetyl-2-deoxy-D-glucopyranosyl nitrate, **150**, in anhydrous acetonitrile at 0°C with one molar equivalent of benzenethiol and Hünigs base (diisopropylamine, DIPEA) as shown in Fig 3.9.<sup>131</sup>

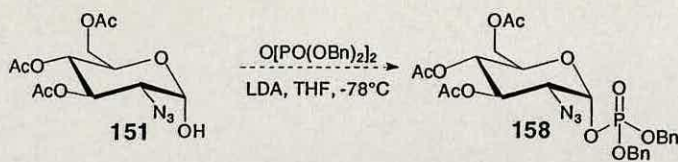


**Fig 3.9 :** Conversion of the anomeric nitro group to an alcohol using Hünigs base.

This reaction was unreliable and did not proceed efficiently. It proved difficult to obtain consistent results with this method and yields ranged from 63% to 0% for product, **151**.

An alternative, more robust process for generating the desired species **151** was found. This method involved treating a solution of the azido-nitrate sugar, **150**, in dioxane, with a solution of sodium nitrite in water. After stirring at 80°C for 3 hours under an atmosphere of nitrogen the desired sugar, **151**, was recovered in 63% yield, Fig 3.7.<sup>132</sup>

The monosaccharide, **151**, was then treated with a 1.8M solution of LDA in 1:1:1 heptane/THF/ethylbenzene at -78°C with the subsequent *in situ* addition of tetrabenzyl pyrophosphate, Fig 3.10.<sup>82</sup>



**Fig 3.10** : Attempted phosphorylation of 2-azido-3,4,6-tri-O-acetyl-2-deoxy-D-glucopyranose.

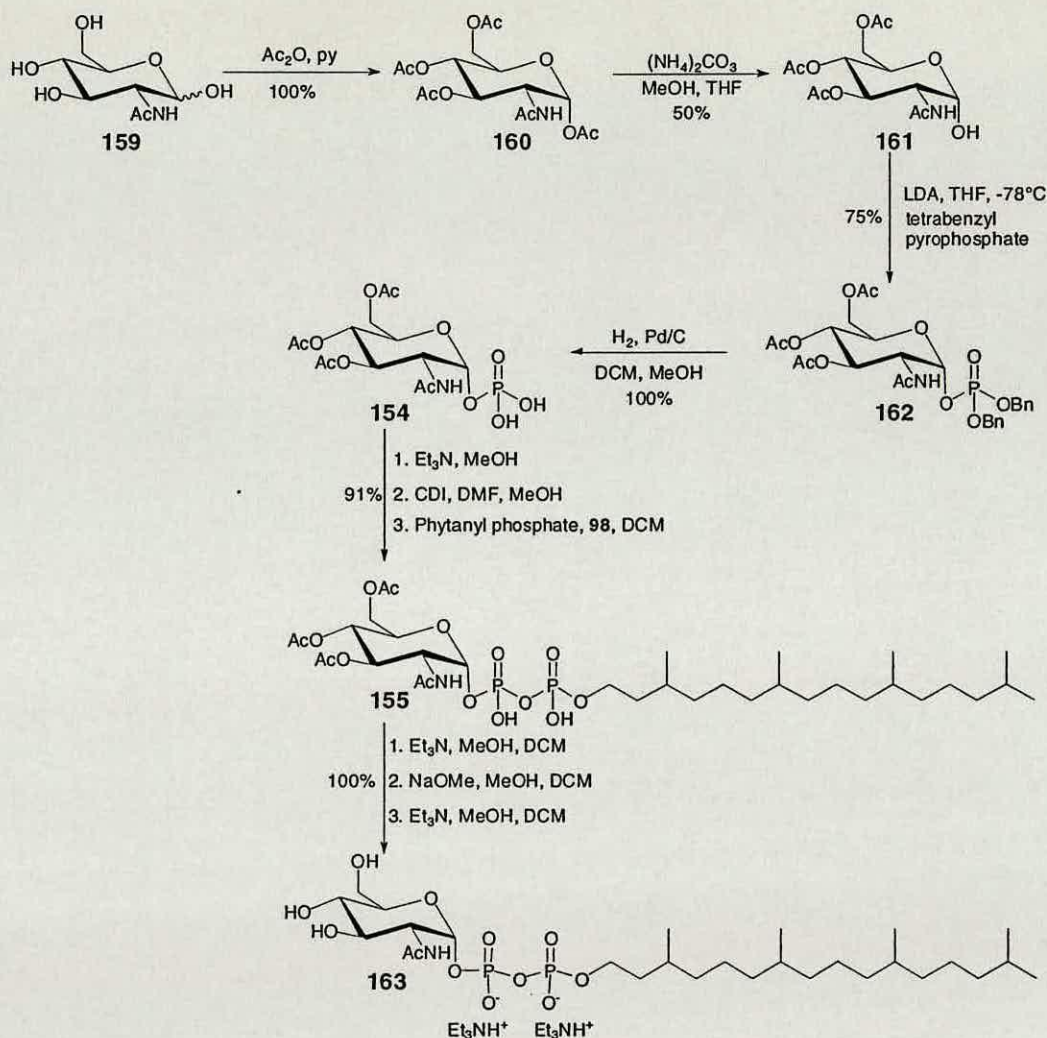
This reaction, however, failed to generate any of the desired glycosyl phosphate dibenzyl ester, **158**, and the compounds recovered from the crude reaction mixture could not be identified.

The phosphorylation method of Perich and Johns was also attempted in the hope that the di-*tert*-butyl glycosyl phosphate, **152**, could be prepared, Fig 3.7.

A solution of the sugar, **151**, in anhydrous THF was treated with *N,N'*-di-*tert*-butyl diisopropylphosphoramidite and 1-*H*-tetrazole with the subsequent *in situ* addition of mCPBA.<sup>102</sup> This method also failed to produce any of the phosphorylated sugar, with only unreacted starting materials isolated.

In view of the low yields of the first two steps of the synthetic route outlined in Fig 3.7 and the difficulty experienced in attempts to synthesise the sugar phosphate, an alternative method to generate the lipid-linked monosaccharide was considered. A synthesis of **163** from *N*-acetyl-D-glucosamine, **159**, was proposed and this is outlined in Fig 3.11.



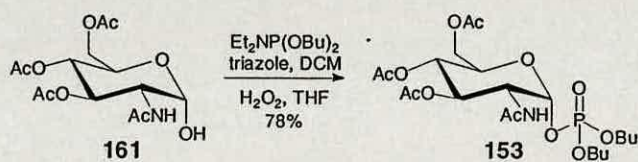


**Fig 3.11 :** The synthesis of *P*<sub>1</sub>-phytanyl-*P*<sub>2</sub>-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl pyrophosphate.

The synthesis of the lipid-linked monosaccharide, **163**, began with the peracetylation of commercially available *N*-acetyl-D-glucosamine, **159**. The sugar was stirred in a mixture of acetic anhydride and pyridine under an atmosphere of nitrogen at room temperature for 16 hours and the desired 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranose, **160**, was isolated in quantitative yield.<sup>133</sup> Selective 1-*O*-deacetylation of the per-acetylated glucosamine, **160**, was achieved by treating a solution of the sugar with an excess of ammonium carbonate.<sup>134</sup> The anomeric deprotection proceeded smoothly affording a moderate, 50%, yield of the monosaccharide, **161**, Fig 3.11. Although the NMR data obtained for **161** was

consistent with the predicted structure it was noted that the melting point obtained for this compound did not agree with that reported in the literature.

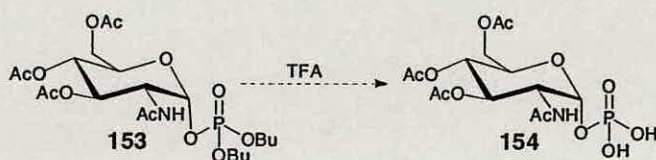
The method developed by Wong *et al.* to generate glycosyl phosphites and phosphates of a number of sugars using dibenzyl *N,N'*-diethylphosphoramidite and triazole followed by oxidation with hydrogen peroxide was discussed in section 2.2.1.<sup>103</sup> This procedure was used to synthesise the di-*tert*-butyl sugar phosphate, **153**, as shown in Fig 3.12.



**Fig 3.12 :** The synthesis of the di-*tert*-butyl glycosyl phosphate, **153**.

A solution of the hemiacetal, **161**, in anhydrous DCM was treated with 1,2,4-triazole and *N,N'*-di-*tert*-butyl diethylphosphoramidite and stirred under an atmosphere of nitrogen. The solution was cooled to  $-78^{\circ}\text{C}$  and treated with 30% hydrogen peroxide solution.<sup>103</sup> This resulted in 78% yield of the desired di-*tert*-butyl glycosyl phosphate, **153**, Fig 3.12.

The final stage in the preparation of the monosaccharide for coupling to the lipid was deprotection of the di-*tert*-butyl glycosyl phosphate, **153**. Initially a solution of the glycosyl phosphate, **153**, in anhydrous DCM was treated with TFA for 10 minutes in order to remove the di-*tert*-butyl protecting groups, Fig 3.13.<sup>35</sup>



**Fig 3.13 :** Attempted deprotection of the di-*tert*-butyl phosphate using TFA.

Unfortunately, this reaction failed to produce the desired glycosyl phosphate, **154**. A number of products were formed but purification of the crude reaction mixture and

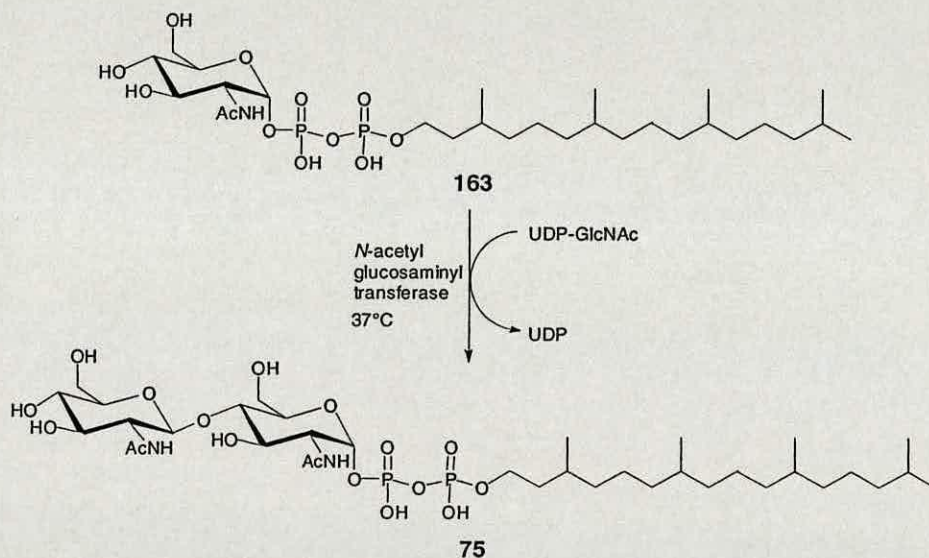


subsequent analysis did not result in identification of any of these side-products. Despite treating a solution of sugar **153** in anhydrous DCM with a variety of acids such as acetic acid, citric acid and TFA, at differing concentrations and for different reaction times, it proved impossible to successfully deprotect the di-*tert*-butyl glycosyl phosphate.

Consequently, an alternative phosphorylation procedure was investigated. The 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranose, **161**, was dissolved in anhydrous THF and treated with a 1.8 M solution of LDA in 1:1:1 heptane/THF/ethylbenzene at  $-78^{\circ}\text{C}$  with the subsequent *in situ* addition of tetrabenzyl pyrophosphate.<sup>82</sup> This reaction afforded selectively the  $\alpha$ -glycosyl phosphate dibenzyl ester, **162**, in 75% yield, Fig 3.11. Hydrogenolysis of the benzyl groups was achieved in the presence of 10% palladium on charcoal under an atmosphere of hydrogen gas to give the deprotected glycosyl phosphate, **154**, in quantitative yield, Fig 3.11.<sup>82</sup> Conversion of phosphate, **154**, to its triethylammonium salt was followed by treatment with *N,N'*-carbonyldiimidazole in DMF to form an intermediate imidazole-carbonyl phosphate anhydride.<sup>82</sup> The addition of methanol to quench any excess *N,N'*-carbonyldiimidazole was followed by the addition of phytanyl phosphate, **98**, to afford the phytanyl pyrophosphoryl monosaccharide, **155**, in 91% yield.<sup>82</sup> Saponification of the protected pyrophosphate derivative using sodium methoxide gave the acceptor substrate, **163**, in quantitative yield.<sup>82</sup>

### 3.5. Enzyme-catalysed synthesis of phytanyl-linked chitobiose.

The final stage of this project was to use the phytanyl-linked glucosamine acceptor substrate, **163**, in an enzyme-catalysed synthesis of phytanyl-linked chitobiose, **75**, Fig 3.14.



**Fig 3.14 :** Enzyme-catalysed conversion of the lipid-linked monosaccharide into phytanyl-linked chitobiose.

The lipid-linked monosaccharide, **163**, was incubated with radiolabelled UDP-[ $^{14}\text{C}$ ]-GlcNAc (0.1  $\mu\text{Ci/mL}$ ) and crude microsomal fractions (1.0-2.0 mg) of *Saccharomyces cerevisiae* in a pH 7.5 buffer solution (1.0 mL) comprising 50.0 mM TrisHCl, 5.0 mM  $\text{MgCl}_2$ , 10.0 mM mercaptoethanol and 0.5% Triton X-100 at 37°C for 1h. The aqueous phase was removed after centrifugation. The organic phase was washed twice with water and incorporation of radioactivity into organic soluble material was measured by scintillation counting. Evidence for the activity of the enzyme was provided by expressing it with the same cell strains that had previously been used within the research group.<sup>82 87 85</sup> A comparison of the results from these tests with material that is known to be active confirmed that the enzyme used in the conversion of **163** to **75** was active.

Dr Ines Sprung attempted this enzyme-catalysed reaction but unfortunately none of the desired phytanyl pyrophosphoryl chitobioside, **75**, was obtained; only unreacted



starting materials were recovered from the reaction mixture. From this, it would appear that the phytanyl-linked monosaccharide, **163**, is not a suitable acceptor substrate for the first glycosyltransferase (*N*-acetylglucosaminyltransferase) in the biosynthetic pathway to *N*-linked glycoproteins.

### 3.6. Conclusion.

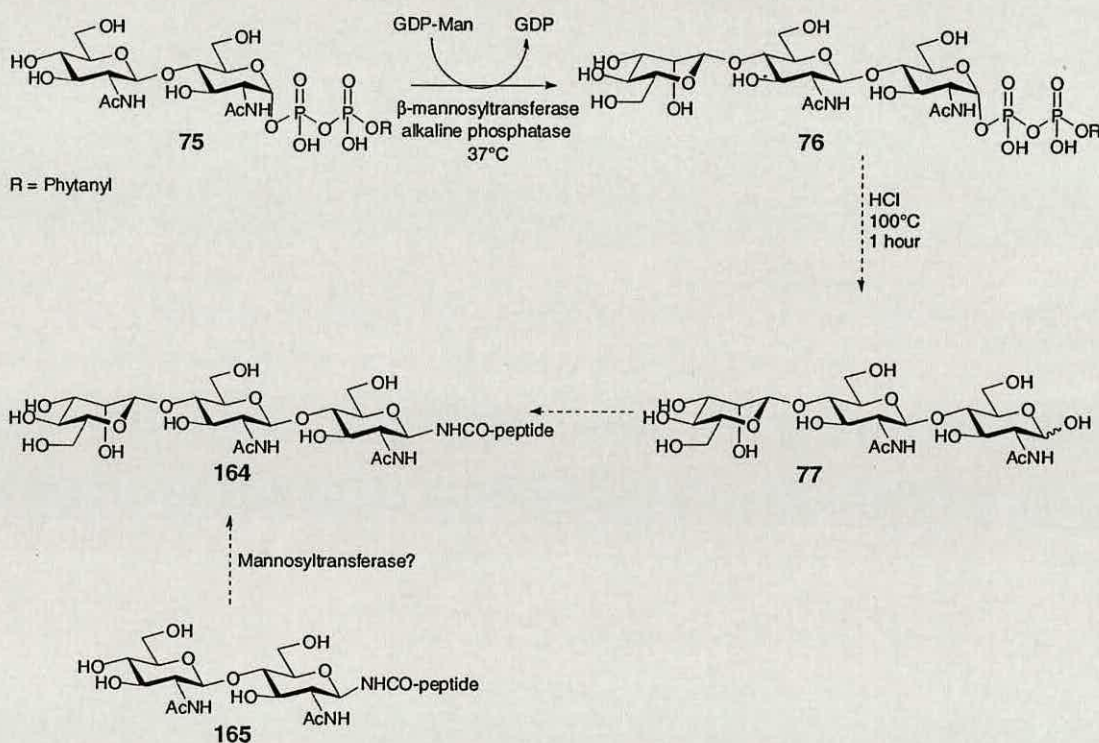
The synthesis of phytanyl-linked chitobiose, **75** (Fig 3.4), from commercially available chitobiose octaacetate, **70** (Fig 3.4), was successfully achieved, using methods that were previously developed in the group.<sup>35, 82</sup> Enzymatic transformation of this substrate was performed to generate a phytanyl-linked trisaccharide, **76** (Fig 3.5), which will be used in future studies involving ALG2.

A new method for preparing phytanyl-linked glucosamine, **163** (Fig 3.11) has also been developed. The aim of devising a cheap, practical and robust chemical synthesis of a lipid-linked monosaccharide has been fulfilled. The yield obtained for each step in this synthetic route was excellent, with the exception of the anomeric deacetylation of peracetylated glucosamine. The low yield of this reaction is counteracted by the ease with which large quantities of 1-*O*-deacetylated glucosamine can be prepared. Although the attempted enzyme-catalysed transformation of phytanyl-linked glucosamine, **163**, to phytanyl-linked chitobiose failed (Fig 3.14), it shows that the phytanyl-linked monosaccharide is not a suitable acceptor substrate for the first glycosyltransferase (*N*-acetylglucosaminyltransferase) in the biosynthetic pathway to *N*-linked glycoproteins.



### 3.7. Synthesis of orthogonally-protected chitobiose.

The ultimate focus of our research was to find simple, practical methods for the synthesis of *N*-linked glycoproteins. Currently, it is possible to synthesise phytanyl-linked chitobiose, **75**, and subsequent enzymatic transformation of this acceptor substrate allows for the preparation of a phytanyl-linked trisaccharide, **76**, as illustrated in Fig 3.15.<sup>82</sup>

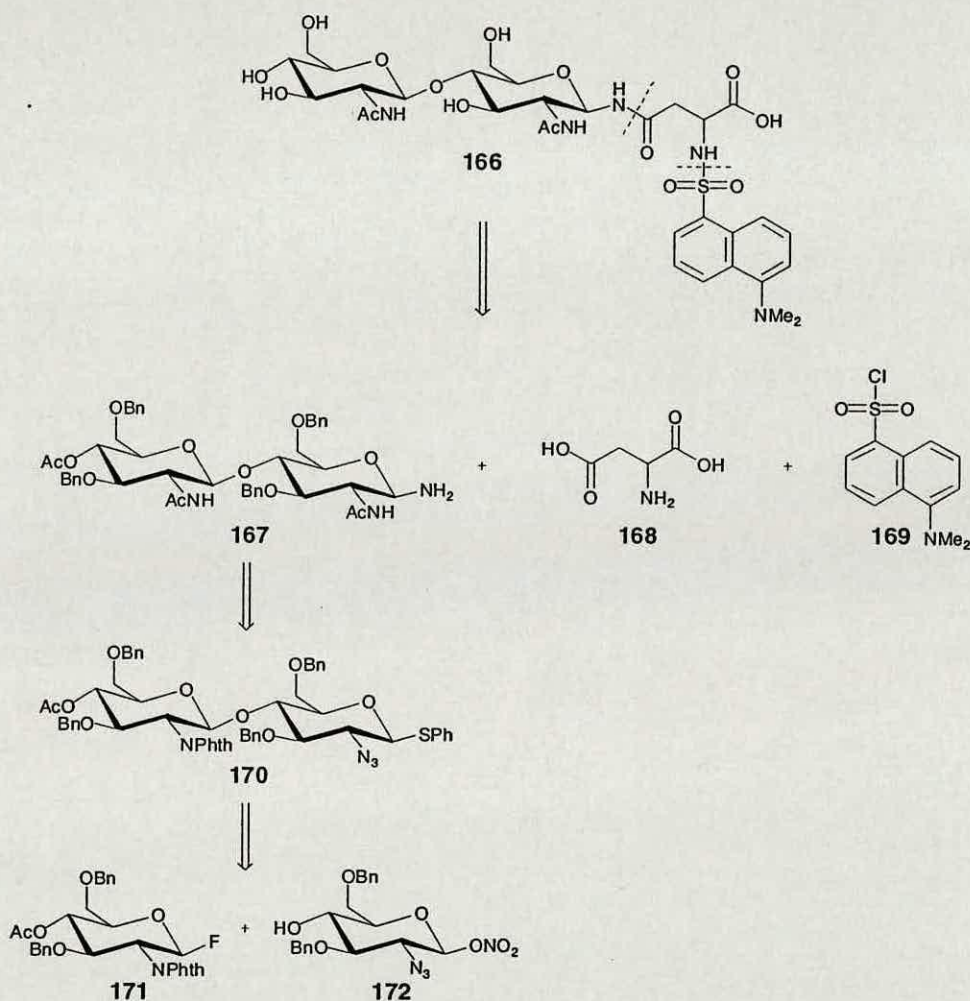


**Fig 3.15 :** *Synthesis of an N-linked trisaccharide, Manβ1-4GlcNAcβ1-4GlcNAc.*

Acid hydrolysis of the lipid-linked trisaccharide, **76**, results in the isolation of the β-mannosyl-linked trisaccharide, Manβ1-4GlcNAcβ1-4GlcNAc, **77**, Fig 3.15.<sup>82</sup> However, direct attachment of this trisaccharide to a peptide, *via* an asparagine residue, is not an easy task. Thus, this research aimed to synthesise an *N*-linked chitobiose glycoprotein, **165**, and then, using enzyme technology, transform it into an *N*-linked trisaccharide, **164**, Fig 3.15. Work in the group has focussed on finding the β-mannosyltransferase needed to catalyse the process. The purpose of my work in this project was to chemically synthesise an orthogonally protected chitobiose

derivative and to attach a fluorescently-labelled asparagine residue. This substrate would then be used in the search for a mannosyltransferase to complete the synthesis of an *N*-linked trisaccharide glycoprotein.

In order to devise a practical synthetic route to the fluorescently labelled asparagine-linked chitobiose, **166**, a retrosynthetic analysis was carried out as depicted in Fig 3.16.



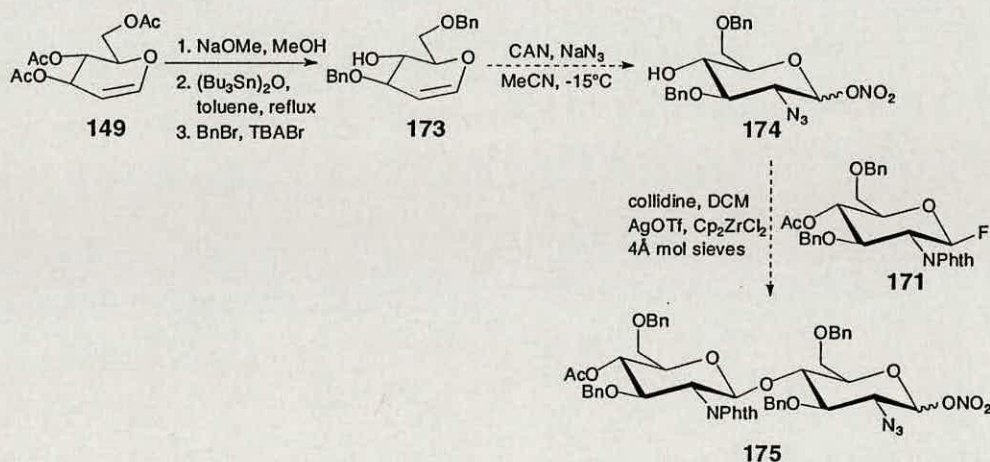
**Fig 3.16 :** Retrosynthetic analysis of the target compound generates commercially available dansyl chloride, **169**, aspartic acid, **168** and orthogonally protected monosaccharides, **171** and **172**.

The target compound, **166**, can be cleaved at two sites, as illustrated in Fig 3.16, to generate accessible synthetic equivalents **167**, **168** and **169**. These synthetic



equivalents include commercially available dansyl chloride, **169**, and aspartic acid, **168**, which can be coupled together to produce the fluorescent amino acid residue. The third synthetic equivalent created in this retrosynthetic analysis is an orthogonally protected chitobiose derivative bearing an amine residue at the anomeric position, **167**. Functional group conversions and disconnection of the  $\beta$ 1-4 linkage of the disaccharide generates two readily available, orthogonally protected monosaccharides, **171** and **172**.

On the basis of this information, a synthetic route to the chitobiose derivative **175** was devised as shown in Fig 3.17. By choosing a suitable protection strategy, it would be possible to selectively react different areas of the chitobiose derivative so that it could also be used in the synthesis of more complex oligosaccharides.



**Fig 3.17 :** Proposed synthesis of orthogonally protected chitobiose.

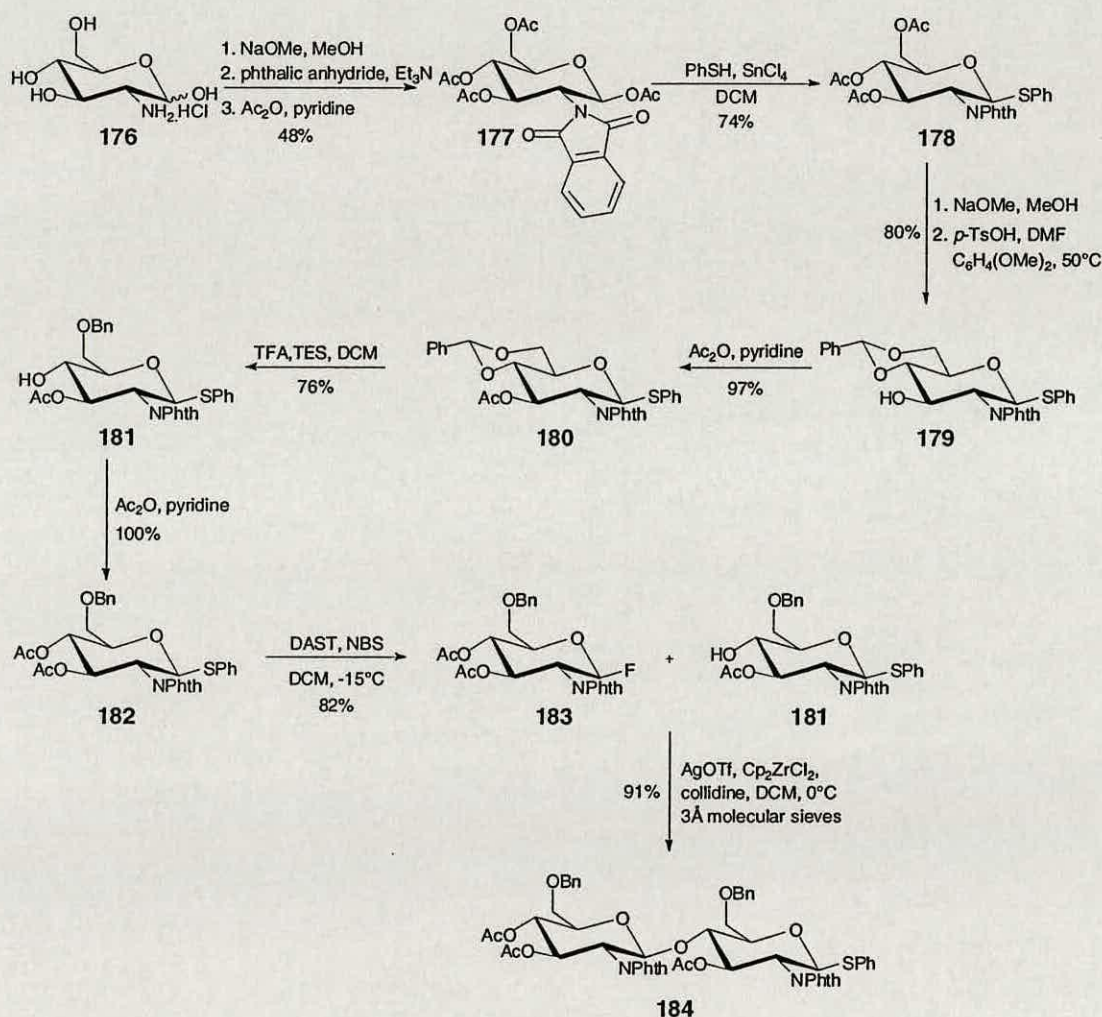
Work commenced with the synthesis of 3,6-*O*-dibenzyl-D-glucal, **173**, using a method developed by Danishefsky *et al.*, Fig 3.17.<sup>135</sup> A solution of tri-*O*-acetyl-D-glucal, **149**, in anhydrous methanol was treated with sodium methoxide and stirred under an atmosphere of nitrogen for three hours. The crude reaction mixture was purified to generate quantitative yield of the totally deprotected glucal. The resulting D-glucal was dissolved in anhydrous toluene and heated at reflux with *bis*-tributyltin oxide using a Dean-Stark apparatus for twenty hours. The reaction mixture was cooled to allow the addition of benzyl bromide (BnBr) and tetrabutylammonium bromide (TBABr) and then heated at reflux for a further sixteen hours to afford a

dark brown residue containing a number of products. Despite repeated attempts to isolate the desired 3,6-*O*-dibenzyl-D-glucal, **173**, from the crude reaction mixture, it proved to be impossible to remove tin side-products from the fraction containing the desired compound.

Consequently, it was not possible to synthesise **174** by the route shown in Fig 3.17 so that it could be used as an acceptor in the assembly of the disaccharide bearing orthogonally protected 2-amino functional groups. However, work had already commenced on the synthesis of a donor component for the coupling reaction as shown in Fig 3.18. It was decided that both the donor and acceptor monosaccharides required for the coupling reaction would be prepared sequentially by extending the route to the donor molecule, since it would be less time consuming and more efficient to synthesise both the donor and acceptor components using just one synthetic route.



The new target disaccharide, **184**, would bear an *N*-phthalimido protecting group at the 2 and 2' position of the disaccharide, Fig 3.18.



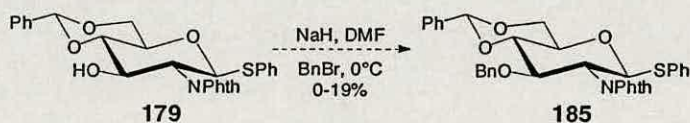
**Fig 3.18 : Synthesis of orthogonally protected chitobiose derivative.**

The synthesis of disaccharide **184** began with the one-pot, three-step acetylation and *N*-phthalimido protection of commercially available glucosamine hydrochloride, **176**. A solution of glucosamine hydrochloride, **176**, in anhydrous methanol was treated with sodium methoxide and stirred under an atmosphere of nitrogen. Addition of phthalic anhydride and triethylamine generated a sticky white solid, which upon reaction with acetic anhydride and pyridine afforded the desired sugar, **177**, in 48% yield.<sup>131</sup> Although this reaction does not produce high yields, it can be performed on a large scale and so significant amounts of **177** can be prepared.



The next step required replacement of the anomeric acetate group with the more versatile thiophenyl functionality. Activation of **177** with the Lewis acid, tin tetrachloride, with subsequent *in situ* addition of benzenethiol resulted in 74% yield of 2-phthalimido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **178**.<sup>131</sup>

The orthogonal protection strategy was further implemented by tethering the hydroxy groups at positions 4 and 6 in a benzylidene acetal. Saponification of **178** with sodium methoxide in anhydrous methanol resulted in the deprotection of the acetyl groups at positions 3, 4 and 6. Subsequent treatment of a solution of the trihydroxy sugar in anhydrous DMF with benzaldehyde dimethyl acetal and catalytic toluene sulfonic acid afforded the 4,6-*O*-benzylidene acetal, **179**, in 80% yield.<sup>131</sup> Although the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for **179** were consistent with the quoted literature, it was noteworthy that the  $[\alpha]_{\text{D}}$  value obtained did not agree with published results. The next step required protection of the free hydroxy group at position 3. Attempts were made to attach a benzyl group at this site by treating a solution of **179** in anhydrous DMF at 0°C with sodium hydride followed by the addition of benzyl bromide, Fig 3.19.<sup>136</sup>



**Fig 3.19 :** Attempted benzylation of the unprotected hydroxy group at position 3.

This reaction failed to generate any product on a number of occasions and when the benzyl protected sugar, **185**, was eventually isolated, it was in only a 19% yield. In order to improve the consistency and increase the yield of product an acetyl protecting group was employed. To a solution of **179** in anhydrous DCM was added acetic anhydride and pyridine. This process successfully generated an almost quantitative yield of the 3-*O*-acetyl protected sugar, **180**, Fig 3.18.

Reductive ring-opening of the 4,6-*O*-benzylidene acetal was next carried out as depicted in Fig 3.18. A triethylsilane (TES)-trifluoroacetic acid (TFA) system was

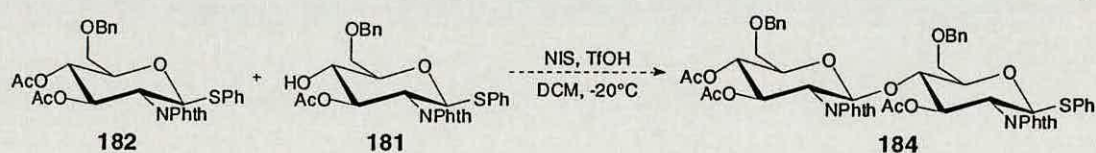


chosen for this reaction because of its reported selective and high-yielding conversions of 4,6-*O*-benzylidene-protected carbohydrates to the corresponding 6-*O*-benzyl-4-hydroxy derivatives.<sup>137</sup> A solution of the 4,6-*O*-benzylidene acetal, **180**, in anhydrous DCM was cooled to 0°C and treated with TES and TFA to generate the desired product **181** in 76% yield, Fig 3.18.

The 2-phthalimido-2-deoxy-3-*O*-acetyl-6-benzyl-1-thiophenyl-β-D-glucopyranoside, **181**, thus generated was divided into two portions. One aliquot was used as the acceptor in the coupling reaction to create protected chitobiose. The remaining sugar was transformed into the donor component for the coupling reaction.

The free 4-hydroxy position of **181** now required protection and again an acetyl group was chosen for this task. A solution of **181** in anhydrous DCM was treated with acetic anhydride and pyridine. The 4-*O*-acetyl protected monosaccharide, **182**, was recovered in quantitative yield.

In order to reduce the number of steps in the synthetic route to the disaccharide an attempt was made to use thioglycoside, **182**, as the glycosyl donor, Fig 3.20.<sup>138</sup>



**Fig 3.20 :** The coupling of donor **182** and acceptor **181** in the presence of NIS and trifluoromethane sulfonic acid.

Coupling of **182** and **181** in the presence of *N*-iodosuccinimide (NIS) and trifluoromethane sulfonic acid in DCM at -20°C failed to produce the disaccharide, **184**, and the species recovered from the crude reaction mixture could not be identified. Further activation of the donor molecule was deemed necessary in order to achieve a successful coupling reaction.

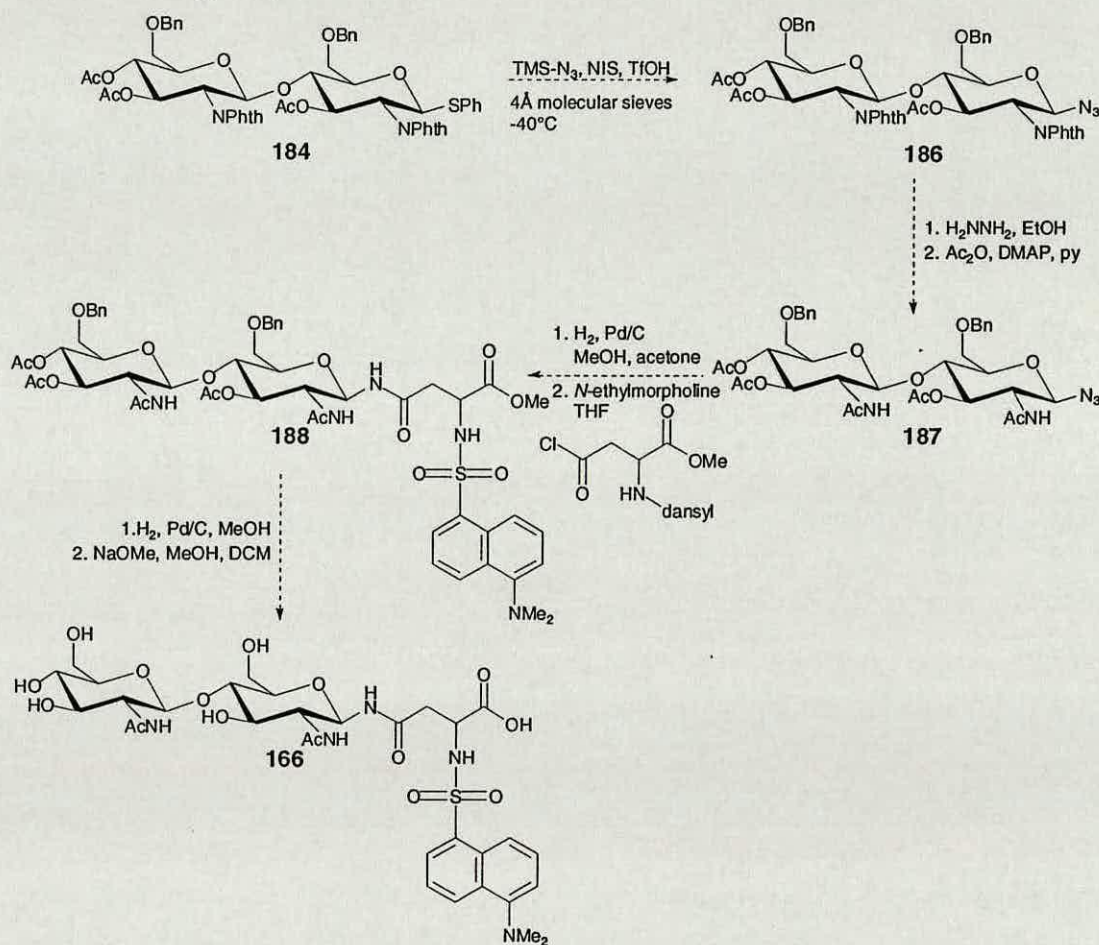
Consequently, the thioglycoside, **182**, was converted to a glycosyl fluoride using a method reported by Nicolaou *et al.*<sup>139</sup> A solution of the thioglycoside, **182**, in

anhydrous DCM was cooled to  $-15^{\circ}\text{C}$  whereupon diethylaminosulfur trifluoride (DAST) and *N*-bromosuccinimide (NBS) were added to the reaction mixture. This reaction resulted in an 82% yield of the glycosyl fluoride, **183**, Fig 3.18.

The coupling of glucosamine derivative, **181**, with glycosyl fluoride, **183**, was carried out under Mukaiyama-Suzuki<sup>140</sup> conditions. The disaccharide **184** was formed in 91% yield by treating a solution of silver triflate, zirconocene dichloride, 2,6-collidine and 3Å molecular sieves in anhydrous DCM with a solution of both donor and acceptor sugars in anhydrous DCM.<sup>141</sup> Evidence for the formation of a  $\beta$ -glycosidic linkage was observed in the  $^1\text{H}$  NMR of the disaccharide where a doublet at  $\delta_{\text{H}}$  5.39 had a spin coupling constant of 8.4 Hz. All other analytical data obtained for **184** were satisfactory with the exception of the elemental analysis. Although the expected %C for this compound was 64.91%, elemental analysis found only 61.78% C. Both %H and %N obtained for this compound were in agreement with the expected values.



Once the orthogonally protected chitobiose derivative had been synthesised it was to be attached to a fluorescently-labelled aspartic acid derivative and then deprotected as shown in Fig 3.21.



**Fig 3.21** : *Proposed synthesis of fluorescently-labelled asparagine-linked chitobiose.*

The first stage in this process involved introduction of a nitrogen group at the anomeric position. A solution of the disaccharide, **184**, in anhydrous DCM containing 4Å molecular sieves was cooled to  $-40^\circ\text{C}$  before adding trimethylsilyl azide, NIS and catalytic triflic acid, Fig 3.21.<sup>138</sup> This reaction failed to generate the desired product and only unreacted starting material was recovered from the reaction mixture. Unfortunately, due to time constraints, it was not possible to attach the anomeric azide and then complete the synthesis of the fluorescently-labelled asparagine-linked chitobiose derivative depicted in Fig 3.21.

### 3.8. Conclusion.

The synthesis of an orthogonally protected chitobiose derivative, **184** (Fig 3.18), has been achieved. This work has formed the basis for the synthesis of a fluorescently-labelled asparagine-linked chitobiose substrate, **166** (Fig 3.21), that will be used to find a  $\beta$ -mannosyltransferase capable of directly mannosylating *N*-linked chitobiose.

### 3.9. Future Work.

The synthesis of an asparagine-linked chitobiose residue will allow research into the availability of an enzyme that could be used in the synthesis of an asparagine-linked trisaccharide.

An alternative means of introducing an azide at the anomeric position is described in the literature whereby thioglycoside **184**, Fig 3.21, would be treated with trimethylsilyl azide and the Lewis acid, tin tetrachloride, in DCM.<sup>142</sup> Having performed their function as activating and  $\beta$ -directing groups, the phthalamide moieties could be removed by treatment with hydrazine, leading to a diamine which could be directly acetylated to afford the diacetamide, **187**, Fig 3.21.<sup>141</sup> Reduction of the azide to an anomeric amine could be achieved by hydrogenolysis using 10% palladium on charcoal as catalyst under an atmosphere of hydrogen gas. Subsequent treatment of the amine with *N*-ethylmorpholine in THF and an aspartic acid residue bearing a dansyl probe should generate the *N*-linked disaccharide, **188**, Fig 3.21.<sup>142</sup> Finally, removal of all the chitobiose protecting groups will generate the target compound, **166**, Fig 3.21.



## 4. Experimental.

### 4.1. General Experimental.

#### 4.1.1. Instrumentation.

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on Bruker AC250, WH360 or Varian Gemini 200 instruments. Both  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were referenced to residual protic solvents i.e.  $\text{CDCl}_3$  ( $\delta_{\text{H}}$  7.3, s;  $\delta_{\text{C}}$  77.0, t),  $(\text{CD}_3)_2\text{SO}$  ( $\delta_{\text{H}}$  2.5, q;  $\delta_{\text{C}}$  39.7, m) and  $\text{CD}_3\text{OD}$  ( $\delta_{\text{H}}$  4.8, s, 3.4, q;  $\delta_{\text{C}}$  49.0, m). Chemical shifts ( $\delta_{\text{H}}$ ,  $\delta_{\text{C}}$ ) are recorded in parts per million (ppm) and coupling constants ( $J$ ) are measured in Hertz (Hz) and quoted to the nearest 0.5 Hz.

Infrared spectroscopy was recorded on a Perkin Elmer Paragon 1000 FT-IR spectrophotometer with the frequencies ( $\nu$ ) measured in wavenumbers ( $\text{cm}^{-1}$ ). Infrared spectra were recorded as thin films on sodium chloride plates.

Melting points were obtained on a Gallenkamp melting point apparatus and are quoted in degrees Celsius ( $^{\circ}\text{C}$ ) and uncorrected.

Optical rotations were performed in dichloromethane on an Optical Activity PolAAR 2 AA series automatic polarimeter.

Elemental analysis (CHN) was performed using a Perkin Elmer 2400 CHN Elemental Analyser.

Fast atom bombardment (FAB) mass spectrometry was performed in positive ion mode using a Kratos MS50TC instrument from a nitrobenzyl alcohol or THIOG matrix. Values are quoted in daltons.

#### **4.1.2. Chromatography.**

Analytical thin layer chromatography (TLC) was carried out on Merck aluminium backed plates coated with silica gel 60 F<sub>254</sub>, 0.25mm. The components were detected using ultra-violet light (254 nm), or visualised with ammonium molybdate and anisaldehyde dips.

Column chromatography was carried out using either the appropriate sized parallel-sided column filled with silica gel 60H (Merck, particle size 0.04-0.063 mm, 230-400 mesh) or a FLASH12I chromatography module with pre-packed cartridge system (biotage company).

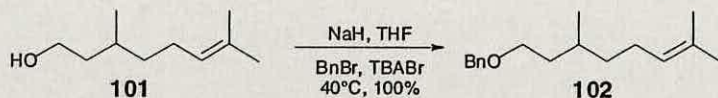
#### **4.1.3. Solvents and Reagents.**

All solvents and reagents were standard laboratory grade and used as supplied unless otherwise stated. Tetrahydrofuran (THF) was pre-dried over sodium wire and distilled from sodium benzophenone ketal while dichloromethane (DCM) was distilled from calcium hydride. All other solvents were purchased as anhydrous grade.



## 4.2. Synthesis of lipid analogues of dolichyl phosphate.

### 8-Benzyloxy-2,6-dimethyloct-2-ene, **102**.<sup>96</sup>

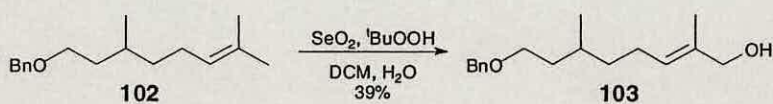


A stirred solution of citronellol, **101**, (20.0 g, 100.0 mmol) in anhydrous THF (80.0 mL) was treated with a 60% dispersion of sodium hydride in mineral oil (5.5 g, 200.0 mmol) and the grey suspension was stirred under N<sub>2</sub> at room temperature for 1h. Benzyl bromide (15.2 mL, 100.0 mmol) and tetrabutylammonium iodide (1.3 g, 3.4 mmol) were added to the reaction mixture which was then stirred at 40°C for 4h under N<sub>2</sub>. The reaction, containing a thick white precipitate, was cooled to room temperature and treated with water (100.0 mL), causing the white solid to dissolve. The solution was extracted with ethyl acetate (3 x 100.0 mL) and the combined organic layers were washed with brine (100.0 mL) and dried over anhydrous MgSO<sub>4</sub>. Filtration, followed by concentration *in vacuo* yielded a dark yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **102**, (31.5 g, 100%) as a pale yellow oil.

R<sub>f</sub> (petroleum ether:ethyl acetate, 6:4) 0.65; HRMS (FAB) 247.2065 ([M+H]<sup>+</sup>, C<sub>17</sub>H<sub>27</sub>O requires 247.2062); LRMS (FAB) 247 (C<sub>17</sub>H<sub>27</sub>O), 107 (C<sub>7</sub>H<sub>7</sub>O), 91 (C<sub>7</sub>H<sub>7</sub>); δ<sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 7.64-7.51 (5H, m, ArH), 5.35 (1H, tm, *J* 7.1 Hz, CH=C(CH<sub>3</sub>)<sub>2</sub>), 4.76 (2H, s, PhCH<sub>2</sub>O), 3.77 (2H, td, *J* 7.0, 2.4 Hz, BnOCH<sub>2</sub>), 2.27-2.22 (2H, br m, CH<sub>2</sub>), 1.94 and 1.86 (2 x 3H, 2 x s, 2 x CH<sub>3</sub>), 1.76-1.35 (5H, br m, CH, 2 x CH<sub>2</sub>), 1.15 (3H, d, *J* 6.4 Hz, CH(CH<sub>3</sub>)); δ<sub>C</sub> (CDCl<sub>3</sub>, 63MHz) 138.6 and 131.0 (qC), 128.2 (2 x ArCH), 127.5 (2 x ArCH), 127.3 and 124.7 (CH), 72.8 and 68.6 (PhCH<sub>2</sub>OCH<sub>2</sub>), 37.1 and 36.6 (CH<sub>2</sub>), 29.4 (CH(CH<sub>3</sub>)), 25.6 (CH<sub>3</sub>), 25.3 (CH<sub>2</sub>), 19.4 and 17.5 (CH<sub>3</sub>); ν<sub>max</sub> (film)/cm<sup>-1</sup> 2926 (CH), 1454 (Ph), 1097 (BnOCH<sub>2</sub>).



## 8-Benzyloxy-2,6-dimethyloct-2-en-1-ol, **103**.<sup>96</sup>

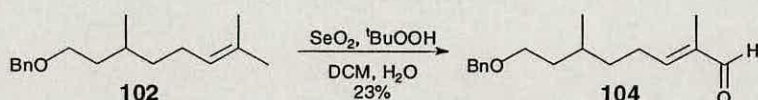


A stirred solution of 8-benzyloxy-2,6-dimethyloct-2-ene, **102**, (12.4 g, 50.4 mmol) in DCM (120.0 mL) was treated with *tert*-butyl hydroperoxide (23.4 mL, 100.0 mmol), selenium dioxide (167.7 mg, 1.5 mmol) and water (2.0 mL). The reaction mixture was stirred for 16h under N<sub>2</sub> at room temperature. The pale pink solution was cooled to 0°C in an ice bath and carefully treated with a saturated solution of sodium metabisulfite (150.0 mL). A peroxide test at this point proved negative and so the resulting white solution was concentrated *in vacuo*. The concentrate was extracted with ethyl acetate (3 x 100.0 mL), the organic layers combined, washed with brine (100.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a dark yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **103**, (5.2 g, 39%) as a pale yellow oil.

R<sub>f</sub> (petroleum ether:ethyl acetate, 8:2) 0.14; HRMS (FAB) 263.2012 ([M+H]<sup>+</sup>, C<sub>17</sub>H<sub>27</sub>O<sub>2</sub> requires 263.2011); LRMS (FAB) 263 (C<sub>17</sub>H<sub>27</sub>O<sub>2</sub>), 261 (C<sub>17</sub>H<sub>25</sub>O<sub>2</sub>), 245 (C<sub>17</sub>H<sub>24</sub>O), 91 (C<sub>7</sub>H<sub>7</sub>); δ<sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 7.57-7.46 (5H, m, ArH), 5.59 (1H, tm, *J* 7.2 Hz, CH=C(CH<sub>3</sub>)<sub>2</sub>), 4.71 (2H, s, PhCH<sub>2</sub>O), 4.27 (2H, s, CH<sub>2</sub>OH), 3.71 (2H, td, *J* 6.2, 2.3 Hz, BnOCH<sub>2</sub>), 2.25-2.22 (2H, br m, CH<sub>2</sub>), 2.09 (1H, br s, CH<sub>2</sub>OH), 1.86 (3H, s, CH<sub>3</sub>), 1.71-1.33 (5H, br m, CH, 2 x CH<sub>2</sub>), 1.11 (3H, d, *J* 6.5 Hz, CH(CH<sub>3</sub>)); δ<sub>C</sub> (CDCl<sub>3</sub>, 63MHz) 138.4 and 134.4 (qC), 128.2 (2 x ArCH), 127.4 (2 x ArCH), 127.3 and 126.2 (CH), 72.7, 68.4, 68.2, 36.6 and 36.4 (CH<sub>2</sub>), 29.3 (CH(CH<sub>3</sub>)), 24.8 (CH<sub>2</sub>), 19.3 and 13.4 (CH<sub>3</sub>); ν<sub>max</sub> (film)/cm<sup>-1</sup> 3412 (OH), 2986 (CH), 1454 (Ph), 1097 (BnOCH<sub>2</sub>).



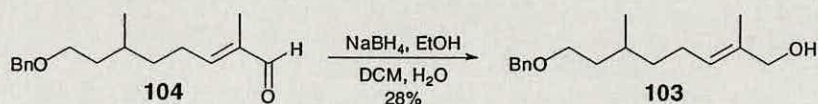
## 8-Benzyloxy-2,6-dimethyl-2-octenal, **104**.<sup>96</sup>



A stirred solution of 8-benzyloxy-2,6-dimethyloct-2-ene, **102**, (12.4 g, 50.4 mmol) in DCM (120.0 mL) was treated with *tert*-butyl hydroperoxide (23.4 mL, 100.0 mol), selenium dioxide (167.7 mg, 1.5 mmol) and water (2.0 mL). The reaction mixture was stirred for 16h under N<sub>2</sub> at room temperature. The pale pink solution was cooled to 0°C in an ice bath and carefully treated with a saturated solution of sodium metabisulfite (150.0 mL). A peroxide test at this point proved negative and so the resulting white solution was concentrated *in vacuo*. The concentrate was extracted with ethyl acetate (3 x 100.0 mL), the organic layers combined, washed with brine (100.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a dark yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **104**, (3.0 g, 23%) as a pale yellow oil.

R<sub>f</sub> (petroleum ether:ethyl acetate, 8:2) 0.36; HRMS (FAB) 261.1857 ([M+H]<sup>+</sup>, C<sub>17</sub>H<sub>25</sub>O<sub>2</sub> requires 261.1855); LRMS (FAB) 261 (C<sub>17</sub>H<sub>25</sub>O<sub>2</sub>), 107 (C<sub>7</sub>H<sub>7</sub>O), 91 (C<sub>7</sub>H<sub>7</sub>), δ<sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 9.37 (1H, s, CHO), 7.39-7.25 (5H, m, ArH), 6.45 (1H, td, *J* 7.4, 1.3 Hz, CH=C(CH<sub>3</sub>)<sub>2</sub>), 4.49 (2H, s, PhCH<sub>2</sub>O), 3.50 (2H, td, *J* 6.9, 2.2 Hz, BnOCH<sub>2</sub>), 1.73 (3H, s, CH<sub>3</sub>), 1.67-1.20 (7H, br m, CH, 3 x CH<sub>2</sub>), 0.92 (3H, d, *J* 6.5 Hz, CH(CH<sub>3</sub>)); δ<sub>C</sub> (CDCl<sub>3</sub>, 63MHz) 195.1 (CHO), 154.8 (CH=C(CH<sub>3</sub>)), 138.5 and 130.9 (qC), 128.1 (2 x ArCH), 127.4 (2 x ArCH), 127.3 (ArCH), 72.8, 68.3, 37.0 and 36.4 (CH<sub>2</sub>), 29.4 (CH(CH<sub>3</sub>)), 25.5 (CH<sub>2</sub>), 19.4 and 19.2 (CH<sub>3</sub>).

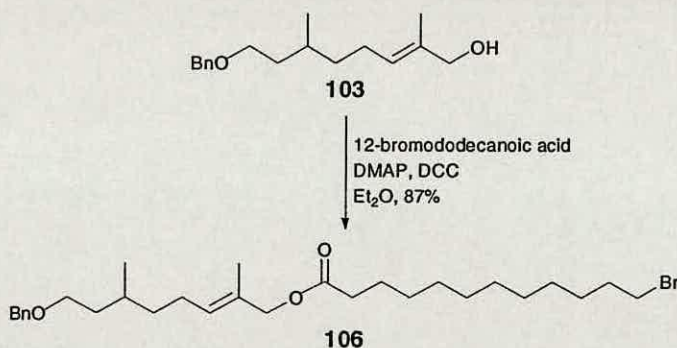
### 8-Benzyloxy-2,6-dimethyloct-2-en-1-ol, **103**.<sup>96</sup>



A stirred solution of 8-benzyloxy-2,6-dimethyl-2-octenal, **104**, (2.31 g, 8.9 mmol) in a 4:1 mixture of MeOH:DCM (15.0 mL) was treated with a solution of sodium borohydride (0.4 g, 10.0 mmol) in water (10.0 mL). The reaction was stirred under an atmosphere of N<sub>2</sub> at room temperature for 16h. The solution was concentrated *in vacuo* and the resulting residue dissolved in diethyl ether (10.0 mL). The organic layer was separated, washed with 0.1 M aq HCl (15.0 mL), water (15.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **103**, (0.65 g, 28%) as a clear oil.

Data as for **103** above.

### 8-Benzyloxy-2,6-dimethyloct-2-en-1-yl 12-bromododecanoate, **106**.

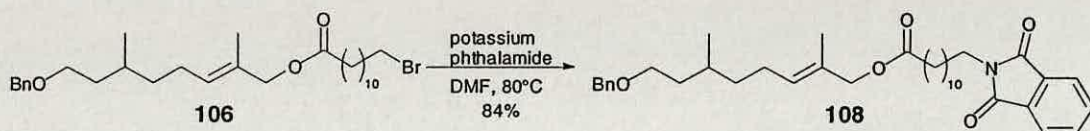


A stirred solution of 8-benzyloxy-2,6-dimethyloct-2-en-1-ol, **103**, (11.0 g, 41.9 mmol) in anhydrous Et<sub>2</sub>O (140.0 mL) was treated with 12-bromododecanoic acid (11.7 g, 41.9 mmol), DMAP (0.5 g, 4.2 mmol) and DCC (9.5 g, 46.1 mmol). A white precipitate rapidly developed and this mixture was stirred for 16h under N<sub>2</sub> at room temperature. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* to yield a yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **106**, (19.0 g, 87%) as a clear oil.



$R_f$  (petroleum ether:ethyl acetate, 9:1) 0.39; HRMS (FAB) 523.2781, 525.2706 ( $[M+H]^+$ ,  $C_{29}H_{48}O_3Br$  requires 523.2787, 525.2708); LRMS (FAB) 523 ( $C_{29}H_{48}O_3Br$ ), 279 ( $C_{12}H_{22}O_2Br$ ), 245 ( $C_{12}H_{22}Br$ ), 154 ( $C_{10}H_{18}O$ ), 91 ( $C_7H_7$ );  $\delta_H$  ( $CDCl_3$ , 250MHz) 7.65-7.54 (5H, m, ArH), 5.72 (1H, tm,  $J$  7.2 Hz,  $CH=C(CH_3)$ ), 4.79 (2H, s,  $PhCH_2O$ ), 4.74 (2H, s,  $CH_2OC=O$ ), 3.79 (2H, td,  $J$  6.8, 2.2 Hz,  $BnOCH_2$ ), 3.69 (2H, t,  $J$  6.9 Hz,  $CH_2Br$ ), 2.61 (2H, t,  $J$  7.5 Hz,  $OC=OCH_2$ ), 2.40-2.28 (2H, br m,  $CH_2$ ), 2.27-2.19 (2H, m,  $CH_2$ ), 1.91 (3H, s,  $CH_3$ ), 1.78-1.52 (21H, br m, CH, 10 x  $CH_2$ ), 1.18 (3H, d,  $J$  6.5 Hz,  $CH(CH_3)$ );  $\delta_C$  ( $CDCl_3$ , 63MHz) 173.7 (CO), 138.5 and 130.8 (qC), 129.8 (CH), 128.2 (2 x ArCH), 127.5 (2 x ArCH), 127.4 (CH), 72.8, 69.9, 68.5, 37.1, 36.5, 36.4, 34.3, 33.9, 32.7 and 29.5 ( $CH_2$ ), 29.3 ( $CH(CH_3)$ ), 29.2, 29.1, 29.0, 28.6, 28.0, 25.1 and 24.9 ( $CH_2$ ), 19.4 and 13.8 ( $CH_3$ );  $\nu_{max}$  (film)/ $cm^{-1}$  2986 (CH), 1725 (OC=O), 1454 (Ph), 1167 ( $CH_2Br$ ), 1097 ( $BnOCH_2$ ).

#### 8-Benzyloxy-2,6-dimethyloct-2-en-1-yl 12-*N*-phthalimidododecanoate, **108**.

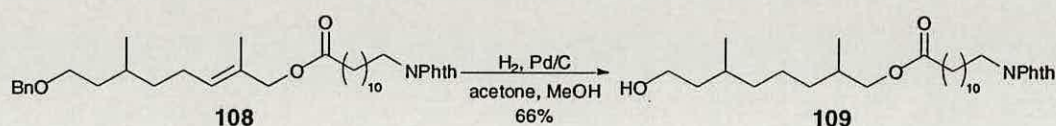


A stirred solution of 8-benzyloxy-2,6-dimethyloct-2-en-1-yl 12-bromododecanoate, **106**, (3.8 g, 7.3 mmol) in anhydrous DMF (50.0 mL) was treated with potassium phthalimide (2.7 g, 14.6 mmol). The reaction mixture was heated to 80 °C and stirred for 16h at this temperature under  $N_2$ . The orange solution was diluted with ethyl acetate (30.0 mL) and washed with water (3 x 30.0 mL). The combined aqueous layers were back extracted with ethyl acetate (20.0 mL). The organic layers were combined, washed with brine (50.0 mL), dried over anhydrous  $MgSO_4$ , filtered and concentrated *in vacuo* to yield an orange oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **108**, (3.6 g, 84%) as a clear oil.



$R_f$  (petroleum ether:ethyl acetate, 9:1) 0.17; HRMS (FAB) 590.3849 ( $[M+H]^+$ ,  $C_{37}H_{52}O_5N$  requires 590.3846); LRMS (FAB) 590 ( $C_{37}H_{52}O_5N$ ), 346 ( $C_{20}H_{28}O_4N$ ), 91 ( $C_7H_7$ );  $\delta_H$  ( $CDCl_3$ , 250MHz) 8.15-7.93 (4H, m, ArH, NPhth), 7.62-7.53 (5H, m, ArH), 5.69 (1H, tm,  $J$  7.2 Hz,  $CH=C(CH_3)$ ), 4.77 (2H, s,  $PhCH_2O$ ), 4.71 (2H, s,  $CH_2OC=O$ ), 3.93 (2H, t,  $J$  7.3 Hz,  $CH_2NPhth$ ), 3.76 (2H, td,  $J$  6.7, 2.4 Hz,  $BnOCH_2$ ), 2.56 (2H, t,  $J$  7.6 Hz,  $OC=OCH_2$ ), 2.38-2.26 (2H, br m,  $CH_2$ ), 2.18-2.06 (2H, m,  $CH_2$ ), 1.97-1.83 (2H, m,  $CH_2$ ), 1.91 (3H, s,  $CH_3$ ), 1.71-1.39 (19H, br m, CH, 9 x  $CH_2$ ), 1.15 (3H, d,  $J$  6.5 Hz,  $CH(CH_3)$ );  $\delta_C$  ( $CDCl_3$ , 63MHz) 177.8 and 173.7 (qC), 168.4 (2 x qC), 138.5 (qC), 133.7 (2 x CH), 132.0 (2 x qC), 129.8 (CH), 128.2 (2 x CH), 127.5 (2 x CH), 127.3 (CH), 123.0 (2 x CH), 72.8, 69.9, 68.5, 37.9, 36.5, 34.3, 33.9 and 32.7 ( $CH_2$ ), 29.5 ( $CH(CH_3)$ ), 29.3, 29.0, 28.6, 28.5, 28.0, 26.7, 25.1, 24.9 and 24.6 ( $CH_2$ ), 19.4 and 13.8 ( $CH_3$ );  $\nu_{max}$  (film)/ $cm^{-1}$  2986 (CH), 1712 ( $OC=O$ ), 1618 and 1551 (N-C=O).

### 8-Hydroxy-2,6-dimethyloctyl 12-*N*-phthalimidododecanoate, **109**.



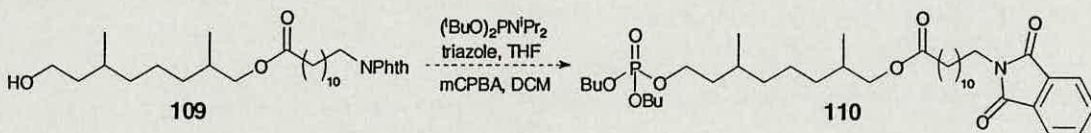
A stirred solution 8-benzyloxy-2,6-dimethyloct-2-en-1-yl 12-*N*-phthalimidododecanoate, **108**, (340.0 mg, 0.5 mmol) in 2:1 acetone : methanol (22.0 mL) was treated with finely divided 10% palladium on charcoal (265.0 mg) as catalyst and placed under an atmosphere of  $N_2$ . The system was evacuated and flushed with hydrogen. This process was repeated twice and the reaction mixture was then allowed to stir under an atmosphere of hydrogen for 16h. The solution was filtered through a pad of celite, which was washed well with acetone (3 x 20.0 mL). The combined filtrate and washings were concentrated *in vacuo* to give a yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **109**, (0.2 g, 66%) as a white solid.

$R_f$  (petroleum ether:ethyl acetate, 1:1) 0.40; Mp 57.0-59.0 °C; HRMS (FAB) 502.3536 ( $[M+H]^+$ ,  $C_{30}H_{48}O_5N$  requires 502.3533); LRMS (FAB) 524



(C<sub>30</sub>H<sub>47</sub>O<sub>5</sub>NNa), 502 (C<sub>30</sub>H<sub>48</sub>O<sub>5</sub>N), 346 (C<sub>20</sub>H<sub>28</sub>O<sub>4</sub>N), 328 (C<sub>20</sub>H<sub>26</sub>O<sub>3</sub>N);  $\delta_{\text{H}}$  (CDCl<sub>3</sub>, 250MHz) 8.04-7.81 (4H, m, ArH, NPhth), 5.09 (1H, br s, OH), 4.05 (1H, m, CH), 3.72 (2H, t, *J* 7.3 Hz, CH<sub>2</sub>NPhth), 2.48-2.37 (2H, m, OC=OCH<sub>2</sub>), 1.80-1.70 (8H, br m, 4 x CH<sub>2</sub>), 1.50-1.32 (23H, br s, CH, 11 x CH<sub>2</sub>), 1.06 (6H, d, *J* 6.4 Hz, 2 x CH(CH<sub>3</sub>));  $\delta_{\text{C}}$  (CDCl<sub>3</sub>, 63MHz) 173.9 (CO), 168.3 (2 x qC), 133.7 (2 x ArCH), 132.0 (2 x qC), 123.0 (2 x ArCH), 69.0, 61.0 and 39.8 (CH<sub>2</sub>), 37.9 (2 x CH<sub>2</sub>), 37.2, 34.9, 34.3 and 33.4 (CH<sub>2</sub>), 32.4 (CH(CH<sub>3</sub>)), 29.4, 29.2, 29.1 and 29.0 (CH<sub>2</sub>), 28.5 (CH(CH<sub>3</sub>)), 26.7 (2 x CH<sub>2</sub>), 24.9 and 24.0 (CH<sub>2</sub>), 19.5 and 16.8 (CH<sub>3</sub>);  $\nu_{\text{max}}$  (film)/cm<sup>-1</sup> 3444 (OH), 2986, 2932 (CH), 1712 (OC=O), 1616 and 1551 (N-C=O).

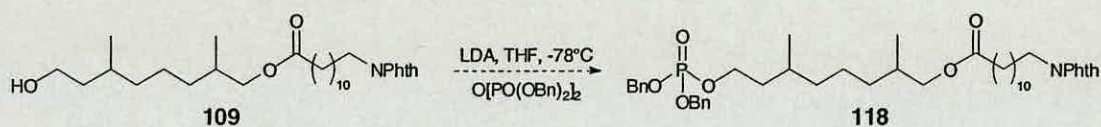
**Di-*tert*-butyl 3,7-dimethyl-8-(12-*N*-phthalimidododecanoyloxy)octyl phosphate, 110.**



A stirred solution of 8-hydroxy-2,6-dimethyloctyl 12-*N*-phthalimidododecanoate, **109**, (0.5 g, 0.9 mmol) in anhydrous THF (10.0 mL) was treated with *N,N'*-di-*tert*-butyl diisopropylphosphoramidite (0.6 mL, 2.0 mmol) and 1,2,4-triazole (0.2 g, 2.9 mmol). The reaction mixture was stirred at room temperature under an atmosphere of N<sub>2</sub> for 3h. The reaction mixture was cooled to 0°C in an ice bath and treated with a solution of mCPBA (0.6 g, 3.2 mmol) in anhydrous DCM (5.0 mL). The reaction mixture was gradually allowed to warm to room temperature and stirred under N<sub>2</sub> for 1h. The reaction mixture was treated with a saturated solution of sodium sulfite (15.0 mL) and stirred for 30 min under N<sub>2</sub>. The solution was extracted with ethyl acetate (3 x 20.0 mL), the organic layers combined, washed with a saturated solution of NaHCO<sub>3</sub> (2 x 20.0 mL) and brine (20.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a yellow oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent but no product was isolated.



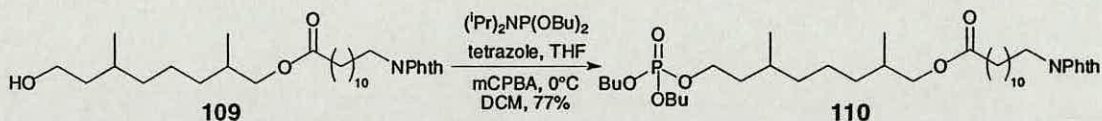
**Dibenzyl 3,7-dimethyl-8-(12-*N*-phthalimidododecanoyloxy)octyl phosphate, 118.**



A stirred solution of 8-hydroxy-2,6-dimethyloctyl 12-*N*-phthalimidododecanoate, **109**, (1.1 g, 2.1 mmol) in anhydrous THF (20.0 mL) was cooled to -78°C and treated with a 1.8 M solution of LDA in 1:1:1 heptane/THF/ethylbenzene (1.7 mL, 2.9 mmol). The reaction mixture was stirred under N<sub>2</sub> for 45 min whereupon a solution of tetrabenzyl pyrophosphate (1.6 g, 2.9 mmol) in anhydrous THF (10.0 mL) was added. The solution was stirred at -78°C for 4h under N<sub>2</sub> after which time it was gradually allowed to warm to room temperature. The reaction was concentrated *in vacuo* to yield a yellow residue. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 7:3, as the eluent to afford the product, **118**, (0.8 g, 49%) as a white solid.

$\delta_{\text{H}}$  (CDCl<sub>3</sub>, 250MHz) 7.99-7.80 (4H, m, ArH, NPhth), 7.46-7.37 (10H, m, ArH), 5.21-5.13 (6H, br m, POCH<sub>2</sub>, 2 x POCH<sub>2</sub>Ph), 4.08-4.02 (2H, m, CH<sub>2</sub>), 3.91 (2H, t, *J* 7.6 Hz, CH<sub>2</sub>NPhth), 2.48 (2H, t, *J* 7.1 Hz, OC=OCH<sub>2</sub>), 1.95-1.88 (8H, m, 4 x CH<sub>2</sub>), 1.57-1.49 (20H, br m, 2 x CH, 9 x CH<sub>2</sub>), 1.14 (6H, d, *J* 6.5 Hz, 2 x CH(CH<sub>3</sub>));  $\delta_{\text{P}}$  (CDCl<sub>3</sub>, 101MHz) -13.3 (s, P=O).

**Di-*tert*-butyl 3,7-dimethyl-8-(12-*N*-phthalimidododecanoyloxy)octyl phosphate, 110.**



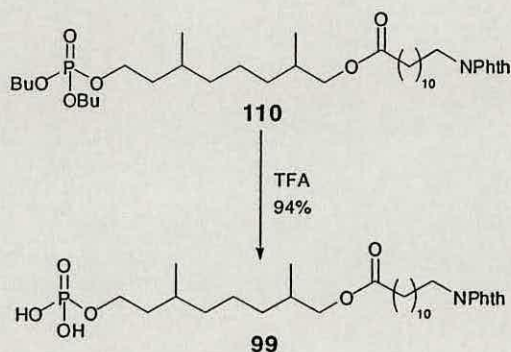
A stirred solution of 8-hydroxy-2,6-dimethyloctyl 12-*N*-phthalimidododecanoate, **109**, (1.0 g, 2.1 mmol) in anhydrous THF (20.0 mL) was treated with *N,N'*-di-*tert*-butyl diisopropylphosphoramidite (1.9 mL, 6.0 mmol) and a 3% solution of 1-*H*-tetrazole in acetonitrile (19.6 mL, 6.6 mmol). A white precipitate developed and the resulting mixture was stirred at room temperature for 3h. The reaction mixture was



cooled to 0°C in an ice bath and treated with a solution of mCPBA (1.7 g, 7.5 mmol) in anhydrous DCM (25.0 mL). The reaction mixture was gradually allowed to warm to room temperature and stirred under N<sub>2</sub> for 1h. The clear, pale blue reaction mixture was treated with a saturated solution of sodium sulfite (40.0 mL) and stirred for 30 min under N<sub>2</sub>. The solution was extracted with ethyl acetate (3 x 50.0 mL), the organic layers combined, washed with a saturated solution of NaHCO<sub>3</sub> (2 x 60.0 mL) and brine (60.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 8:2, as the eluent to afford the product, **110**, (1.1 g, 77%) as a white solid.

R<sub>f</sub> (petroleum ether:ethyl acetate, 7:3) 0.12; Mp 65.0-67.0 °C; HRMS (FAB) 694.4442 ([M+H]<sup>+</sup>, C<sub>38</sub>H<sub>65</sub>O<sub>8</sub>NP requires 694.4448); LRMS (FAB) 716 (C<sub>38</sub>H<sub>64</sub>O<sub>8</sub>NPNa), 694 (C<sub>38</sub>H<sub>65</sub>O<sub>8</sub>NP), 582 (C<sub>30</sub>H<sub>49</sub>O<sub>8</sub>NP), 346 (C<sub>20</sub>H<sub>28</sub>O<sub>4</sub>N); δ<sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 8.13-7.99 (4H, m, ArH, NPhth), 4.23-4.12 (4H, complex m, POCH<sub>2</sub>, CH<sub>2</sub>), 4.10-4.08 (2H, m, CH<sub>2</sub>), 3.95 (2H, t, *J* 7.4 Hz, CH<sub>2</sub>NPhth), 2.56 (2H, t, *J* 7.6 Hz, OC=OCH<sub>2</sub>), 2.05-1.88 (6H, m, 3 x CH<sub>2</sub>), 1.74 (18H, s, 2 x C(CH<sub>3</sub>)<sub>3</sub>), 1.51-1.46 (20H, br m, 2 x CH, 9 x CH<sub>2</sub>), 1.17 (6H, d, *J* 6.5 Hz, 2 x CH(CH<sub>3</sub>)); δ<sub>C</sub> (CDCl<sub>3</sub>, 63MHz) 173.9 (CO), 168.3 (2 x qC), 133.7 (ArCH), 132.6 (2 x qC), 129.9, 127.9 and 123.3 (ArCH), 82.8 and 82.0 (OC(CH<sub>3</sub>)<sub>3</sub>), 69.1, 65.1, 46.5 and 37.9 (CH<sub>2</sub>), 36.9 (2 x CH<sub>2</sub>), 34.2 and 33.4 (CH<sub>2</sub>), 32.4 (CH(CH<sub>3</sub>)), 30.2 (2 x C(CH<sub>3</sub>)<sub>3</sub>), 29.7 (2 x CH<sub>2</sub>), 29.3 (CH(CH<sub>3</sub>)), 29.0 (2 x CH<sub>2</sub>), 28.4, 26.7, 24.9, 23.9 and 22.2 (CH<sub>2</sub>), 19.1 and 16.8 (CH<sub>3</sub>); δ<sub>P</sub> (CDCl<sub>3</sub>, 101MHz) -6.5 (s, P=O); ν<sub>max</sub> (film)/cm<sup>-1</sup> 2986 (CH), 1713 (OC=O), 1168 (P=O) and 984 (P-O-C).

**3,7-Dimethyl-8-(12-*N*-phthalimidododecanoyloxy)octyl phosphate, 99.**

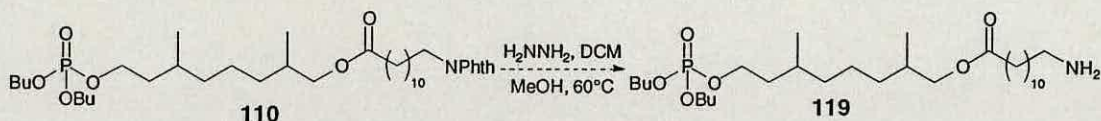


A solution of di-tert-butyl 3,7-dimethyl-8-(12-*N*-phthalimidododecanoyloxy)octyl phosphate, **110**, (109.8 mg, 0.2 mmol) in TFA (5.0 mL, 65.2 mmol) was stirred under N<sub>2</sub> at room temperature for 10 min. The reaction mixture was then concentrated *in vacuo* and the resulting residue was azeotroped with anhydrous toluene (2 x 15.0 mL) to afford the product, **99**, (86.0 mg, 94%) as a white solid.

R<sub>f</sub> (DCM:MeOH:H<sub>2</sub>O:2M aq NH<sub>4</sub>HCO<sub>3</sub>, 6.5:3.5:0.4:0.4) 0.19; HRMS (FAB) 582.3198 ([M+H]<sup>+</sup>, C<sub>30</sub>H<sub>49</sub>O<sub>8</sub>NP requires 582.3196); LRMS (FAB) 626 (C<sub>30</sub>H<sub>48</sub>O<sub>8</sub>NPNa<sub>2</sub>), 604 (C<sub>30</sub>H<sub>47</sub>O<sub>8</sub>NPNa), 582 (C<sub>30</sub>H<sub>49</sub>O<sub>8</sub>NP), 328 (C<sub>20</sub>H<sub>26</sub>O<sub>3</sub>N); δ<sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 7.94-7.77 (4H, m, ArH, NPhth), 7.09 (2H, br s, P(OH)<sub>2</sub>), 4.07-3.82 (4H, complex m, POCH<sub>2</sub>, CH<sub>2</sub>), 3.76 (2H, t, *J* 7.3 Hz, CH<sub>2</sub>NPhth), 2.37 (2H, t, *J* 7.5 Hz, OC=OCH<sub>2</sub>), 1.78-1.65 (6H, m, 3 x CH<sub>2</sub>), 1.45-1.25 (22H, br m, 2 x CH, 10 x CH<sub>2</sub>), 0.98-0.88 (6H, br m, 2 x CH(CH<sub>3</sub>)); δ<sub>C</sub> (CDCl<sub>3</sub>, 63MHz) 173.9 (CO), 168.3 (2 x qC), 133.7 (2 x ArCH), 132.0 (2 x qC), 123.0 (2 x ArCH), 69.1 and 64.9 (CH<sub>2</sub>), 37.9 (2 x CH<sub>2</sub>), 37.3 (CH<sub>2</sub>), 34.2 (2 x CH<sub>2</sub>), 33.5 (CH<sub>2</sub>), 32.4 (CH(CH<sub>3</sub>)), 29.3 (2 x CH<sub>2</sub>), 29.1 (CH(CH<sub>3</sub>)), 28.5 (2 x CH<sub>2</sub>), 26.7 (2 x CH<sub>2</sub>), 24.9 (2 x CH<sub>2</sub>), 23.9 (CH<sub>2</sub>), 19.1 and 16.9 (CH<sub>3</sub>); δ<sub>P</sub> (CDCl<sub>3</sub>, 250MHz) 0.0 (s, P=O); ν<sub>max</sub> (film)/cm<sup>-1</sup> 2929, 2856 (CH), 1711 (OC=O), 1172 (P=O) and 907 (P-O-C).



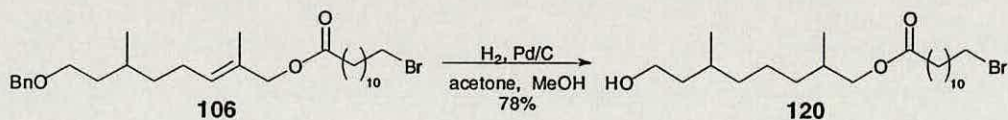
**Di-*tert*-butyl 3,7-dimethyl-8-(12-aminododecanoyloxy)octyl phosphate, 119.**



A stirred solution of di-*tert*-butyl 3,7-dimethyl-8-(12-*N*-phthalimidododecanoyloxy)octyl phosphate, **110**, (200.0 mg, 0.3 mmol) in a 2:1 mixture of anhydrous MeOH and DCM (6.0 mL) was treated with hydrazine hydrate (0.2 mL, 2.8 mmol). The reaction mixture was stirred at 60°C for 2h under an atmosphere of N<sub>2</sub>. The solution was cooled to room temperature and concentrated *in vacuo* to yield a white solid. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 1:1, as the eluent but no product was isolated.

The reaction was repeated using the same quantites of each material as given above, but without heating to 60°C for 2h. Instead the solution was stirred under an atmosphere of N<sub>2</sub> for 16h and then worked up as before. Again no product was recovered from the process.

**8-Hydroxy-2,6-dimethyloctyl 12-bromododecanoate, 120.**



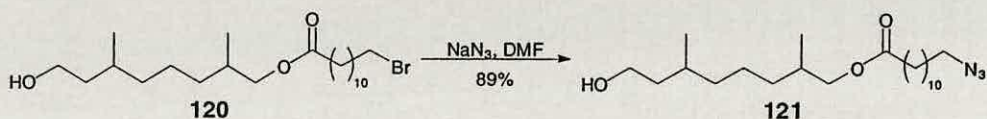
A stirred solution of 8-benzyloxy-2,6-dimethyloct-2-en-1-yl 12-bromododecanoate, **106**, (2.3 g, 4.3 mmol) in 2:1 acetone:methanol (30.0 mL) was treated with finely divided 10% palladium on charcoal (350.0 mg) as catalyst and placed under an atmosphere of N<sub>2</sub>. The system was evacuated and flushed with hydrogen. This process was repeated twice and the reaction mixture was then allowed to stir under an atmosphere of hydrogen for 16h. The solution was filtered through a pad of celite, which was washed well with acetone (3 x 30.0 mL). The combined filtrate and washings were concentrated *in vacuo* to give a yellow oil. This was purified by



column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **120**, (1.5 g, 78%) as a white solid.

$R_f$  (petroleum ether:ethyl acetate, 8:2) 0.18; Mp 43.0–45.0 °C; HRMS (FAB) 457.2289, 459.2201 ( $[M+Na]^+$ ,  $C_{22}H_{43}O_3BrNa$  requires 457.2294, 459.2204); CHN found %C 60.51, %H 9.97; (required %C 60.69, %H 9.89); LRMS (FAB) 457 ( $C_{22}H_{43}O_3BrNa$ ), 245 ( $C_{12}H_{22}Br$ ), 154 ( $C_{10}H_{18}O$ );  $\delta_H$  ( $CDCl_3$ , 250MHz) 6.08 (1H, br s, OH), 3.86–3.80 (2H, m,  $HOCH_2$ ), 3.56 (2H, t,  $J$  6.9 Hz,  $CH_2Br$ ), 2.49 (2H, t,  $J$  7.5 Hz,  $OC=OCH_2$ ), 2.06–1.94 (2H, m,  $CH_2$ ), 1.83–1.65 (4H, m, 2 x  $CH_2$ ), 1.59–1.39 (20H, br m, 2 x CH, 9 x  $CH_2$ ), 1.34–1.23 (4H, m, 2 x  $CH_2$ ), 1.01 (6H, d,  $J$  6.5 Hz, 2 x  $CH(CH_3)$ );  $\delta_C$  ( $CDCl_3$ , 63MHz) 179.4 (CO), 61.1 and 39.7 ( $CH_2$ ), 39.1 (2 x  $CH_2$ ), 37.2 (2 x  $CH_2$ ), 33.9 and 32.7 ( $CH_2$ ), 29.3 ( $CH(CH_3)$ ), 29.2 (2 x  $CH_2$ ), 29.1 (2 x  $CH_2$ ), 28.9, 28.6 and 28.0 ( $CH_2$ ), 27.8 ( $CH(CH_3)$ ), 24.5 and 22.6 ( $CH_2$ ), 22.5 and 19.5 ( $CH_3$ );  $\nu_{max}$  (film)/ $cm^{-1}$  3400 (OH), 2986 (CH), 1709 (OC=O), 1167 ( $CH_2Br$ ).

#### 8-Hydroxy-2,6-dimethyloctyl 12-azidododecanoate, **121**.



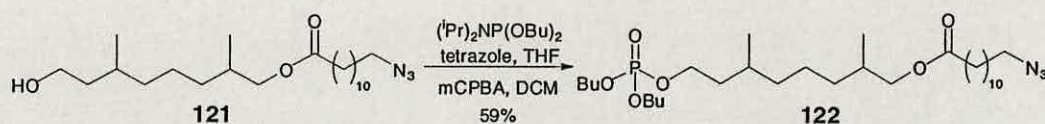
A stirred solution of 8-hydroxy-2,6-dimethyloctyl 12-bromododecanoate, **120**, (3.7 g, 8.4 mmol) in anhydrous DMF (50.0 mL) was treated with sodium azide (1.6 g, 25.3 mmol). The reaction mixture was stirred at room temperature under  $N_2$  for 16h. Water (25.0 mL) was added and the reaction mixture was extracted with DCM (3 x 50.0 mL). The organic layers were combined, washed with brine (70.0 mL), dried over anhydrous  $MgSO_4$ , filtered and concentrated *in vacuo* to yield a pale yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **121**, (3.0 g, 89%) as a clear oil.

$R_f$  (petroleum ether:ethyl acetate, 8:2) 0.22; HRMS (FAB) 398.3380 ( $[M+H]^+$ ,  $C_{22}H_{44}O_3N_3$  requires 398.3383); LRMS (FAB) 398 ( $C_{22}H_{44}O_3N_3$ ), 370 ( $C_{22}H_{44}O_3N$ ),



154 (C<sub>10</sub>H<sub>18</sub>O);  $\delta_{\text{H}}$  (CDCl<sub>3</sub>, 250MHz) 4.18-4.00 (2H, m, CH<sub>2</sub>), 3.88-3.84 (2H, m, CH<sub>2</sub>), 3.45 (2H, t, *J* 6.9 Hz, CH<sub>2</sub>N<sub>3</sub>), 2.51 (2H, t, *J* 7.5 Hz, OC=OCH<sub>2</sub>), 2.01-1.90 (2H, m, CH<sub>2</sub>), 1.85-1.73 (6H, m, 3 x CH<sub>2</sub>), 1.63-1.37 (20H, br m, 2 x CH, 9 x CH<sub>2</sub>), 1.09 (6H, d, *J* 6.6 Hz, 2 x CH(CH<sub>3</sub>));  $\delta_{\text{C}}$  (CDCl<sub>3</sub>, 63MHz) 173.9 (CO), 69.0, 60.9, 51.3, 39.8, 39.7, 37.1, 34.5, 33.8 and 33.5 (CH<sub>2</sub>), 32.4 (CH(CH<sub>3</sub>)), 29.3 and 29.1 (CH<sub>2</sub>), 28.9 (CH(CH<sub>3</sub>)), 28.7, 26.6, 24.9, 24.6, 23.9 and 19.5 (CH<sub>2</sub>), 18.5 and 16.8 (CH<sub>3</sub>);  $\nu_{\text{max}}$  (film)/cm<sup>-1</sup> 3400 (OH), 2986 (CH), 1725 (OC=O).

**Di-*tert*-butyl 3,7-dimethyl-8-(12-azidododecanoyloxy)octyl phosphate, 122.**



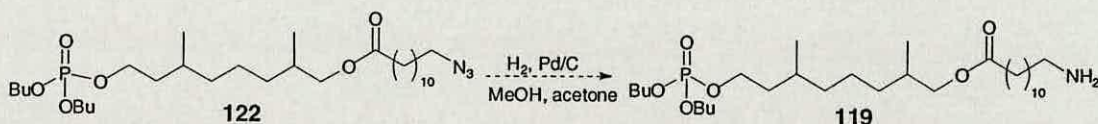
A stirred solution of 8-hydroxy-2,6-dimethyloctyl 12-azidododecanoate, **121**, (2.0 g, 5.1 mmol) in anhydrous THF (20.0 mL) was treated with N,N'-di-*tert*-butyl diisopropylphosphoramidite (4.9 mL, 15.4 mmol) and a 3% solution of 1-*H*-tetrazole in acetonitrile (48.6 mL, 16.4 mmol). A white precipitate developed and the resulting solution was stirred at room temperature for 3h. The reaction mixture was cooled to 0°C and treated with a solution of mCPBA (4.1 g, 18.5 mmol) in anhydrous DCM (25.0 mL). The reaction mixture was gradually allowed to warm to room temperature and stirred under N<sub>2</sub> for 1h. The clear, pale blue reaction mixture was treated with a saturated solution of sodium sulfite (40.0 mL) and stirred for 30min under N<sub>2</sub>. The solution was extracted with ethyl acetate (3 x 50.0 mL), the organic layers were combined, washed with a saturated solution of NaHCO<sub>3</sub> (2 x 60.0 mL) and brine (60.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, (1.8 g, 59%) as a clear oil.

*R<sub>f</sub>* (petroleum ether:ethyl acetate, 9:1) 0.09; HRMS (FAB) 590.4298 ([M+H]<sup>+</sup>, C<sub>30</sub>H<sub>61</sub>O<sub>6</sub>N<sub>3</sub>P requires 590.4298); LRMS (FAB) 590 (C<sub>30</sub>H<sub>61</sub>O<sub>6</sub>N<sub>3</sub>P), 562 (C<sub>30</sub>H<sub>61</sub>O<sub>6</sub>NP), 365 (C<sub>18</sub>H<sub>38</sub>O<sub>5</sub>P);  $\delta_{\text{H}}$  (CDCl<sub>3</sub>, 250MHz) 4.31-4.15 (2H, m, CH<sub>2</sub>), 4.07



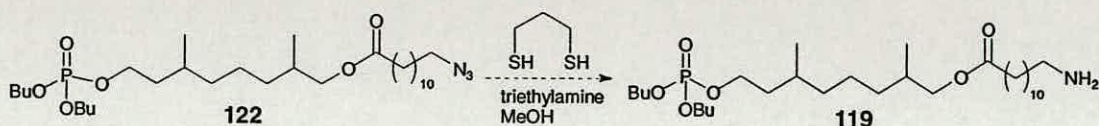
(2H, dd,  $J$  10.7, 6.9 Hz, POCH<sub>2</sub>), 3.49 (2H, t,  $J$  6.9 Hz, CH<sub>2</sub>N<sub>3</sub>), 2.55 (2H, t,  $J$  7.5 Hz, OC=OCH<sub>2</sub>), 2.03-1.94 (2H, m, CH<sub>2</sub>), 1.85-1.79 (6H, m, 3 x CH<sub>2</sub>), 1.75 (18H, s, 2 x C(CH<sub>3</sub>)<sub>3</sub>), 1.61-1.47 (20H, br m, 10 x CH<sub>2</sub>), 1.14 (6H, d,  $J$  4.8 Hz, 2 x CH(CH<sub>3</sub>));  $\delta_C$  (CDCl<sub>3</sub>, 63MHz) 173.9 (CO), 81.8 and 81.7 (OC(CH<sub>3</sub>)<sub>3</sub>), 69.0 and 64.9 (CH<sub>2</sub>), 51.3 (2 x CH<sub>2</sub>), 36.9 (2 x CH<sub>2</sub>), 34.3 (2 x CH<sub>2</sub>), 33.5 (CH<sub>2</sub>), 32.4 (CH(CH<sub>3</sub>)), 29.7 (CH<sub>2</sub>), 29.3 (2 x C(CH<sub>3</sub>)<sub>3</sub>), 29.1 (CH(CH<sub>3</sub>)), 29.0 (2 x CH<sub>2</sub>), 28.7 (CH<sub>2</sub>), 26.6 (2 x CH<sub>2</sub>), 24.9 and 23.9 (CH<sub>2</sub>), 19.2 and 16.7 (CH<sub>3</sub>);  $\delta_P$  (CDCl<sub>3</sub>, 101MHz) -7.8 (s, P=O);  $\nu_{\max}$  (film)/cm<sup>-1</sup> 1725 (OC=O), 1175 (P=O), 997 (POC).

**Di-*tert*-butyl 3,7-dimethyl-8-(12-aminododecanoyloxy)octyl phosphate, 119.**



A stirred solution of di-*tert*-butyl 3,7-dimethyl-8-(12-azidododecanoyloxy)octyl phosphate, **122**, (1.0 g, 1.7 mmol) in 2:1 acetone:methanol (15.0 mL) was treated with finely divided 10% palladium on charcoal (302.0 mg) as catalyst and placed under an atmosphere of N<sub>2</sub>. The system was evacuated and flushed with hydrogen. This process was repeated twice and the reaction mixture was then allowed to stir under an atmosphere of hydrogen for 16h. The solution was filtered through a pad of celite, which was washed well with acetone (3 x 20.0 mL). The combined filtrate and washings were concentrated *in vacuo* to give a yellow oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 1:1, as the eluent but no product was isolated.

**Di-*tert*-butyl 3,7-dimethyl-8-(12-aminododecanoyloxy)octyl phosphate, 119.**



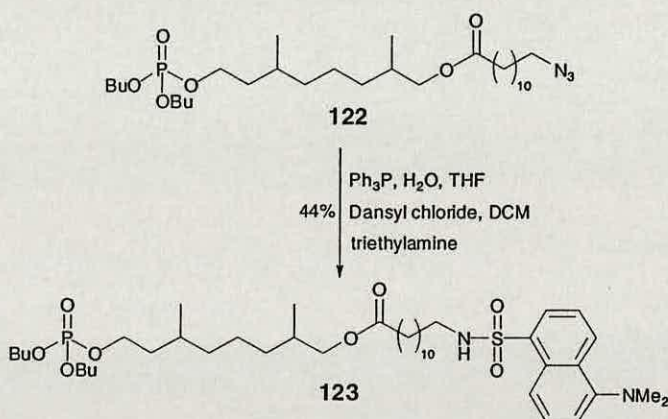
A stirred solution of di-*tert*-butyl 3,7-dimethyl-8-(12-azidododecanoyloxy)octyl phosphate, **122**, (0.5 g, 0.9 mmol) in anhydrous MeOH (5.0 mL) was treated with propane-1,3-dithiol (0.3 mL, 2.5 mmol) and triethylamine (0.4 mL, 2.5mmol). The reaction mixture was stirred under an atmosphere of N<sub>2</sub> for 48h and then



concentrated *in vacuo*. TLC analysis of the crude reaction mixture showed that only starting materials were present. This was confirmed by mass spectral analysis.

$R_f$  (petroleum ether:ethyl acetate, 9:1) 0.09; LRMS (FAB) 590 ( $C_{30}H_{61}O_6N_3P$ ), 562 ( $C_{30}H_{61}O_6NP$ ), 365 ( $C_{18}H_{38}O_5P$ ).

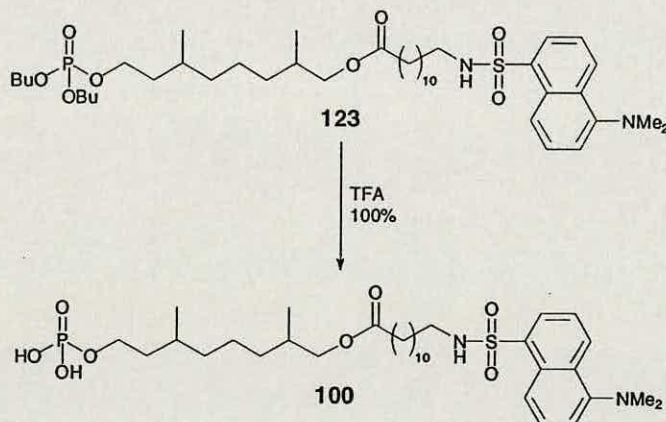
**Di-*tert*-butyl 3,7-dimethyl-8-[12-(4'-dimethylaminonaphthalene-1'-sulfonamido)dodecanoyloxy]octyl phosphate, 123.**



A stirred solution of di-*tert*-butyl 3,7-dimethyl-8-(12-azidododecanoyloxy)octyl phosphate, **122**, (0.6 g, 1.0 mmol) in THF (10.0 mL) and  $H_2O$  (0.1 mL) was treated with triphenylphosphine (1.2 g, 4.6 mmol) and stirred for 16h at room temperature under  $N_2$ . The reaction mixture was concentrated *in vacuo* to yield a white solid. A portion of the white solid (0.3 g, 0.5 mmol) was dissolved in anhydrous DCM (4.0 mL) and treated with triethylamine (65.0  $\mu L$ , 0.5 mmol) and dansyl chloride (130.0 mg, 0.5 mmol). The resulting bright orange reaction mixture was stirred under  $N_2$  at room temperature for 16h. The solution was diluted with  $H_2O$  (5.0 mL) and the organic layer separated. The aqueous layer was extracted with DCM (3 x 5.0 mL). The organic layers were combined, washed with brine (10.0 mL), dried over anhydrous  $MgSO_4$ , filtered and concentrated *in vacuo* to yield a brown oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 8:2, as the eluent to afford the product, **123**, (0.2 g, 44%) as a clear oil.

$R_f$  (DCM:MeOH:H<sub>2</sub>O:2M aq NH<sub>4</sub>HCO<sub>3</sub>, 6.5:3.5:0.4:0.4) 0.77; HRMS (FAB) 796.4874 ( $[M]^+$ , C<sub>42</sub>H<sub>73</sub>O<sub>8</sub>N<sub>2</sub>PS requires 796.4865); LRMS (FAB) 797 (C<sub>42</sub>H<sub>74</sub>O<sub>8</sub>N<sub>2</sub>PS), 590 (C<sub>34</sub>H<sub>58</sub>O<sub>4</sub>N<sub>2</sub>S), 450 (C<sub>24</sub>H<sub>38</sub>O<sub>4</sub>N<sub>2</sub>S);  $\delta_H$  (CDCl<sub>3</sub>, 250MHz) 8.81 (1H, m, ArH), 8.48 (2H, m, 2 x ArH), 7.79 (1H, m, ArH), 7.67 (1H, m, ArH), 7.44 (1H, d,  $J$  6.9 Hz, ArH), 5.10-5.06 (1H, m, NH), 4.29-4.15 (2H, m, CH<sub>2</sub>), 4.12 (2H, dd,  $J$  10.5, 6.8 Hz, POCH<sub>2</sub>), 3.67 (2H, m, CH<sub>2</sub>NH), 3.14 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 2.55 (2H, td,  $J$  7.5 Hz, OC=OCH<sub>2</sub>), 2.04-1.95 (2H, m, CH<sub>2</sub>), 1.90-1.81 (4H, m, 2 x CH<sub>2</sub>), 1.73 (18H, s, 2 x C(CH<sub>3</sub>)<sub>3</sub>), 1.61-1.38 (22H, br m, 2 x CH, 10 x CH<sub>2</sub>), 1.16 (6H, d,  $J$  5.1 Hz, 2 x CH(CH<sub>3</sub>));  $\delta_C$  (CDCl<sub>3</sub>, 63MHz) 173.9 (CO), 165.9, 151.8, 136.6 and 134.6 (ArC), 132.0, 131.8, 128.5, 128.3, 128.1 and 127.2 (ArCH), 81.8 and 81.7 (OC(CH<sub>3</sub>)<sub>3</sub>), 68.9 and 64.9 (CH<sub>2</sub>), 45.3 (N(CH<sub>3</sub>)<sub>2</sub>), 43.2 and 40.1 (CH<sub>2</sub>), 36.9 (2 x CH<sub>2</sub>), 34.2 and 33.4 (CH<sub>2</sub>), 32.4 and 29.7 (CH(CH<sub>3</sub>)), 29.3 (2 x CH<sub>2</sub>), 29.2 (2 x CH<sub>2</sub>), 29.1 (2 x C(CH<sub>3</sub>)<sub>3</sub>), 28.8, 26.8, 26.3, 24.9 and 23.9 (CH<sub>2</sub>), 19.1 and 16.7 (CH<sub>3</sub>);  $\delta_P$  (CDCl<sub>3</sub>, 250MHz) -6.5 (s, P=O);  $\nu_{max}$  (film)/cm<sup>-1</sup> 1712 (OC=O), 1363 (N-S(=O)<sub>2</sub>), 1223 (P=O), 1091 (C-N), 997 (POC).

**3,7-dimethyl-8-[12-(4'-dimethylaminonaphthalene-1'-sulfonamido)dodecanoyloxy]octyl phosphate, 100.**



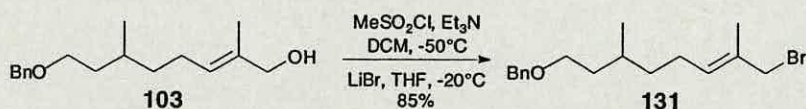
A solution of di-tert-butyl 3,7-dimethyl-8-[12-(4'-dimethylaminonaphthalene-1'-sulfonamido)dodecanoyloxy]octyl phosphate, **123**, (35.0 mg, 0.1 mmol) in TFA (1.4 mL, 18.1 mmol) was stirred under N<sub>2</sub> at room temperature for 10min. The reaction mixture was then concentrated *in vacuo* and the resulting residue was azeotroped



with anhydrous toluene (2 x 10.0 mL) to afford the product, **100**, (30.0 mg, 100%) as a white solid.

$R_f$  (DCM:MeOH:H<sub>2</sub>O:2M aq NH<sub>4</sub>HCO<sub>3</sub>, 6.5:3.5:0.4:0.4) 0.41; HRMS (FAB) 685.3666 ( $[M+H]^+$ , C<sub>34</sub>H<sub>58</sub>O<sub>8</sub>N<sub>2</sub>PS requires 685.3655); LRMS (FAB) 707 (C<sub>34</sub>H<sub>57</sub>O<sub>8</sub>N<sub>2</sub>PSNa), 685 (C<sub>34</sub>H<sub>58</sub>O<sub>8</sub>N<sub>2</sub>PS), 449 (C<sub>24</sub>H<sub>37</sub>O<sub>4</sub>N<sub>2</sub>S), 250 (C<sub>12</sub>H<sub>14</sub>O<sub>2</sub>N<sub>2</sub>S);  $\delta_H$  (CDCl<sub>3</sub>, 250MHz) 8.73 (1H, d,  $J$  8.5 Hz, ArH), 8.50 (1H, d,  $J$  8.7 Hz, ArH), 8.42 (1H, dd,  $J$  7.3, 1.2 Hz, ArH), 7.98 (1H, m, ArH), 7.75 (1H, dd,  $J$  8.8, 1.3 Hz, ArH), 7.40 (1H, d,  $J$  7.1 Hz, ArH), 4.19-3.99 (6H, br m, POCH<sub>2</sub>, 2 x OH, CH<sub>2</sub>), 3.63-3.57 (2H, m, CH<sub>2</sub>NH), 3.06 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 2.51 (2H, t,  $J$  7.1 Hz, OC=OCH<sub>2</sub>), 2.02-1.92 (2H, br m, CH<sub>2</sub>), 1.85-1.75 (6H, br m, 3 x CH<sub>2</sub>), 1.64-1.48 (14H, br s, 2 x CH, 6 x CH<sub>2</sub>), 1.53-1.41 (6H, br m, 3 x CH<sub>2</sub>), 1.13-1.06 (6H, d,  $J$  6.6 Hz, 2 x CH(CH<sub>3</sub>));  $\delta_C$  (CDCl<sub>3</sub>, 63MHz) 174.3 (CO), 163.3, 151.6, 134.6 and 134.3 (ArC), 131.1, 130.0, 129.6, 129.2, 127.9 and 127.0 (ArCH), 69.0 and 63.8 (CH<sub>2</sub>), 45.1 (N(CH<sub>3</sub>)<sub>2</sub>), 42.8 (CH<sub>2</sub>), 39.9 (2 x CH<sub>2</sub>), 37.1 and 34.1 (CH<sub>2</sub>), 33.3 (2 x CH<sub>2</sub>), 32.3 (CH(CH<sub>3</sub>)), 29.1 (2 x CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 28.8 (CH(CH<sub>3</sub>)), 28.7, 26.7, 26.2, 24.7 and 23.8 (CH<sub>2</sub>), 18.8 and 16.5 (CH<sub>3</sub>);  $\delta_P$  (CDCl<sub>3</sub>, 101MHz) 6.3 (s, P=O);  $\nu_{max}$  (film)/cm<sup>-1</sup> 1711 (OC=O), 1364 (N-S(=O)<sub>2</sub>), 1223 (P=O), 1092 (C-N).

### 8-Benzyloxy-1-bromo-2,6-dimethyloct-2-ene, **131**.<sup>96</sup>



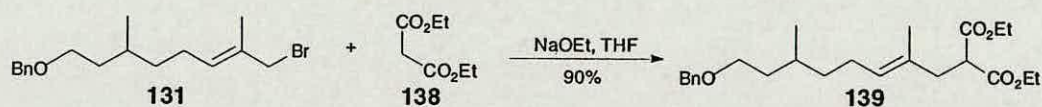
A stirred solution of 8-benzyloxy-2,6-dimethyloct-2-en-1-ol, **103**, (1.0 g, 3.8 mmol) in anhydrous DCM (15.0 mL) was cooled to  $-50^\circ\text{C}$  and treated with triethylamine (0.8 g, 7.6 mmol). The reaction mixture was stirred for 5 minutes under an atmosphere of nitrogen before the addition of methanesulfonyl chloride (0.7 g, 6.5 mmol). A white precipitate developed and this mixture was stirred for 1.5h under N<sub>2</sub> at  $-50^\circ\text{C}$ . The reaction mixture was warmed to  $-20^\circ\text{C}$  and a solution of lithium



bromide (1.2 g, 13.3 mmol) in anhydrous THF (7.0 mL) was added to the flask. After stirring at  $-20^{\circ}\text{C}$  for 2h under an atmosphere of nitrogen, the reaction mixture was poured into ice-water (30.0 mL). The solution was extracted with DCM (3 x 50.0 mL), the organic layers combined, washed with brine (30.0 mL) and water (30.0 mL), dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **131**, (1.1 g, 85%) as a white solid.

$R_f$  (petroleum ether:ethyl acetate, 9:1) 0.68; HRMS (FAB) 323.1018, 325.0992 ( $[\text{M}]^+$ ,  $\text{C}_{17}\text{H}_{24}\text{OBr}$  requires 323.1011, 325.0991); LRMS (FAB) 325 ( $\text{C}_{17}\text{H}_{24}\text{OBr}$ ), 107 ( $\text{C}_7\text{H}_7\text{O}$ ), 91 ( $\text{C}_7\text{H}_7$ );  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 250MHz) 7.35-7.25 (5H, m, ArH), 5.61 (1H, tm,  $J$  7.0 Hz,  $\text{CH}=\text{C}(\text{CH}_3)$ ), 4.49 (2H, s,  $\text{PhCH}_2\text{O}$ ), 3.96 (2H, s,  $\text{CH}_2\text{Br}$ ), 3.53 (2H, td,  $J$  6.5, 1.3 Hz,  $\text{BnOCH}_2$ ), 2.04 (3H, s,  $\text{CH}_3$ ), 1.83-1.62 (4H, br m, 2 x  $\text{CH}_2$ ), 1.58-1.20 (3H, br m,  $\text{CH}_2$ , CH), 0.89 (3H, d,  $J$  6.3 Hz,  $\text{CH}(\text{CH}_3)$ );  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 63MHz) 138.5 (qC), 131.6 (CH), 131.3 (qC), 131.0 (CH), 128.2 (2 x ArCH), 127.5 (2 x ArCH), 72.8, 68.4, 41.8, 36.5 and 36.1 ( $\text{CH}_2$ ), 29.4 ( $\text{CH}(\text{CH}_3)$ ), 25.6 ( $\text{CH}_2$ ), 19.3 and 14.5 ( $\text{CH}_3$ ).

#### Diethyl 2-(8-benzyloxy-2,6-dimethyloct-2-en-1-yl) malonate, **139**.



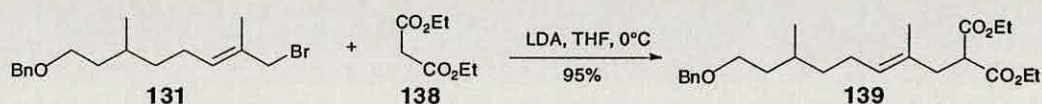
A stirred solution of diethyl malonate, **138**, (150.0 mg, 318.5  $\mu\text{mol}$ ) in anhydrous THF (5.0 mL) was treated with sodium ethoxide (21.7 mg, 318.5  $\mu\text{mol}$ ). The reaction mixture was stirred for 1h under an atmosphere of  $\text{N}_2$  at room temperature before the addition of 8-benzyloxy-1-bromo-2,6-dimethyloct-2-ene, **131**, (103.6 mg, 318.5  $\mu\text{mol}$ ). The reaction mixture was stirred under an atmosphere of  $\text{N}_2$  for 16h. The solution was diluted with water (5.0 mL) and extracted with ethyl acetate (3 x 10.0 mL), the organic layers were combined, washed with brine (15.0 mL), dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a yellow oil. This was



purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **139**, (115.9 mg, 90%) as a clear oil.

$R_f$  (petroleum ether:ethyl acetate, 9:1) 0.30; HRMS (FAB) 405.2640 ( $[M+H]^+$ ,  $C_{24}H_{37}O_5$  requires 405.2640); LRMS (FAB) 405 ( $C_{24}H_{37}O_5$ ), 313 ( $C_{17}H_{30}O_5$ ), 91 ( $C_7H_7$ );  $\delta_H$  ( $CDCl_3$ , 250MHz) 7.39-7.28 (5H, m, ArH), 5.15 (1H, t,  $J$  6.9 Hz,  $CH=C(CH_3)$ ), 4.48 (2H, s,  $PhCH_2O$ ), 4.36 (1H, t,  $J$  7.6 Hz,  $CH(CO_2Et)_2$ ), 4.20-4.04 (4H, m, 2 x  $CO_2CH_2CH_3$ ), 3.47 (2H, td,  $J$  7.2, 2.3 Hz,  $BnOCH_2$ ), 2.55 (2H, d,  $J$  8.2 Hz,  $CH_2$ ), 2.02-1.91 (3H, br m,  $CH_2$ ,  $CH$ ), 1.52 (3H, s,  $CH_3$ ), 1.26-1.14 (7H, br m,  $CH_3$ , 2 x  $CH_2$ ), 0.89-0.85 (6H, m, 2 x  $CH_2CH_3$ );  $\delta_C$  ( $CDCl_3$ , 63MHz) 172.2 and 169.7 (C=O), 139.1 (qC), 131.6 (CH), 130.6 (qC), 128.8 (2 x ArCH), 128.0 (2 x ArCH), 127.9 (CH), 73.3, 69.1 and 61.3 ( $CH_2$ ), 43.6 ( $CH(CO_2Et)_2$ ), 38.9, 37.4 and 37.1 ( $CH_2$ ), 30.0 ( $CH(CH_3)$ ), 25.9 and 25.8 ( $CH_2$ ), 19.9, 17.2, 14.5 and 14.4 ( $CH_3$ );  $\nu_{max}$  (film)/ $cm^{-1}$  2920 (CH), 1715 (OC=O) and 1450 (Ph).

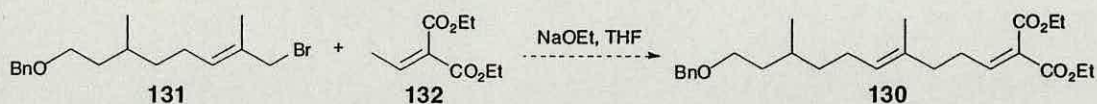
#### Diethyl 2-(8-benzyloxy-2,6-dimethyloct-2-en-1-yl) malonate, **139**.



A stirred solution of diethyl malonate, **138**, (24.6 mg, 153.7  $\mu$ mol) in anhydrous THF (1.0 mL) was cooled to 0°C and treated with a 1.8 M solution of LDA in 1:1:1 heptane/THF/ethylbenzene (80.0  $\mu$ L, 153.7  $\mu$ mol). The reaction mixture was stirred for 45 minutes under an atmosphere of  $N_2$  at 0°C before the addition of 8-benzyloxy-1-bromo-2,6-dimethyloct-2-ene, **131**, (50.0 mg, 153.7  $\mu$ mol). The reaction mixture was warmed to room temperature and stirred under an atmosphere of  $N_2$  for 16h. The solution was diluted with water (5.0 mL) and extracted with ethyl acetate (3 x 10.0 mL), the organic layers were combined, washed with brine (15.0 mL), dried over anhydrous  $MgSO_4$ , filtered and concentrated *in vacuo* to yield a yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **139**, (59.1 mg, 95%) as a clear oil. Analytical data as above.

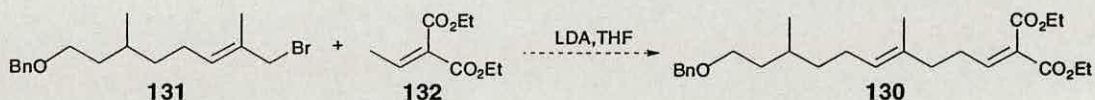


**Diethyl 12-benzyloxy-2-carbethoxy-6,10-dimethyldodec-2,6-dienoate, 130.**



A stirred solution of diethyl ethylidene malonate, **132**, (172.0 mg, 922.5  $\mu\text{mol}$ ) in anhydrous THF (5.0 mL) was treated with sodium ethoxide (62.7 mg, 922.5  $\mu\text{mol}$ ). The reaction mixture was stirred for 1h under an atmosphere of  $\text{N}_2$  at room temperature before the addition of 8-benzyloxy-1-bromo-2,6-dimethyloct-2-ene, **131**, (200.6 mg, 614.7  $\mu\text{mol}$ ). The reaction mixture was stirred under an atmosphere of  $\text{N}_2$  for 16h. The solution was diluted with water (5.0 mL) and extracted with ethyl acetate (3 x 10.0 mL), the organic layers were combined, washed with brine (15.0 mL), dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a yellow oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent but no product was isolated.

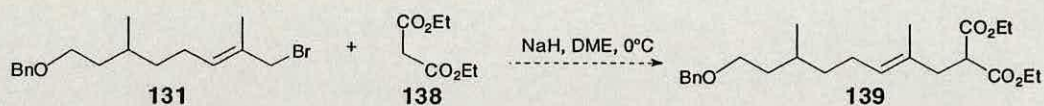
**Diethyl 12-benzyloxy-2-carbethoxy-6,10-dimethyldodec-2,6-dienoate, 130.**



A stirred solution of diethyl ethylidene malonate, **132**, (57.0 mg, 307.0  $\mu\text{mol}$ ) in anhydrous THF (5.0 mL) was cooled to  $0^\circ\text{C}$  and treated with a 1.8 M solution of LDA in 1:1:1 heptane/THF/ethylbenzene (154.0  $\mu\text{L}$ , 307.0  $\mu\text{mol}$ ). The reaction mixture was stirred for 45 minutes under an atmosphere of  $\text{N}_2$  at  $0^\circ\text{C}$  before the addition of 8-benzyloxy-1-bromo-2,6-dimethyloct-2-ene, **131**, (100.0 mg, 307.0  $\mu\text{mol}$ ). The reaction mixture was stirred under an atmosphere of  $\text{N}_2$  for 16h. The solution was diluted with water (5.0 mL) and extracted with ethyl acetate (3 x 10.0 mL), the organic layers were combined, washed with brine (15.0 mL), dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a yellow oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent but no product was isolated.

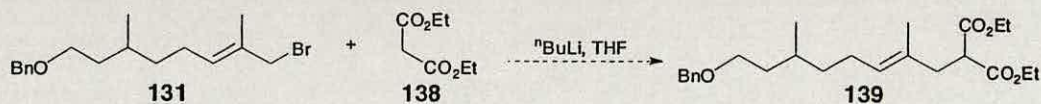


**Diethyl 2-(8-benzyloxy-2,6-dimethyloct-2-en-1-yl) malonate, 139.**



A stirred solution of diethyl malonate, **138**, (49.2 mg, 307.0  $\mu\text{mol}$ ) in anhydrous DME (3.0 mL) was cooled to 0°C and treated with a 60% dispersion of sodium hydride in mineral oil (4.7 mg, 184.0  $\mu\text{mol}$ ). The reaction mixture was stirred at 0°C for 30 min under an atmosphere of  $\text{N}_2$  before the addition of 8-benzyloxy-1-bromo-2,6-dimethyloct-2-ene, **131**, (100.0 mg, 307.0  $\mu\text{mol}$ ). The reaction mixture was gradually allowed to warm to room temperature and stirred under an atmosphere of  $\text{N}_2$  for 16h. The solution was diluted with water (5.0 mL) and extracted with ethyl acetate (3 x 10.0 mL), the organic layers were combined, washed with brine (15.0 mL), dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a yellow oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent but no product was isolated.

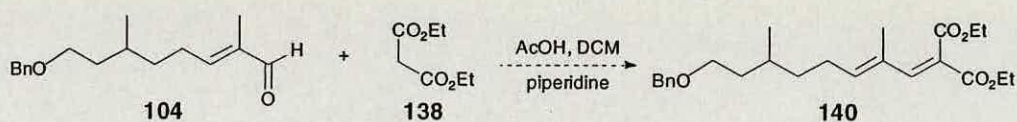
**Diethyl 2-(8-benzyloxy-2,6-dimethyloct-2-en-1-yl) malonate, 139.**



A stirred solution of diethyl malonate, **138**, (24.6 mg, 154.0  $\mu\text{mol}$ ) in anhydrous THF (3.0 mL) was cooled to 0°C and treated with a 1.6 M solution of  $^n\text{BuLi}$  in hexanes (50.0  $\mu\text{L}$ , 76.0  $\mu\text{mol}$ ). The reaction mixture was stirred at 0°C for 30 min under an atmosphere of  $\text{N}_2$  before the addition of 8-benzyloxy-1-bromo-2,6-dimethyloct-2-ene, **131**, (50.0 mg, 154.0  $\mu\text{mol}$ ). The reaction mixture was gradually allowed to warm to room temperature and stirred under an atmosphere of  $\text{N}_2$  for 16h. The solution was diluted with water (5.0 mL) and extracted with ethyl acetate (3 x 10.0 mL), the organic layers were combined, washed with brine (15.0 mL), dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a yellow oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent but no product was isolated.

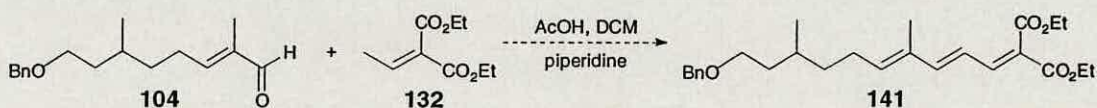


**Diethyl 10-benzyloxy-2-carbethoxy-4,8-dimethyldec-2,4-dienoate, 140.**



A stirred solution of 8-benzyloxy-2,6-dimethyl-2-octenal, **104**, (200.0 mg, 768.0  $\mu\text{mol}$ ) and diethyl malonate, **138**, (135.3 mg, 844.8  $\mu\text{mol}$ ) in anhydrous DCM (5.0 mL) was treated with a solution of acetic acid (8.2 mg, 153.6  $\mu\text{mol}$ ) and piperidine (13.0 mg, 153.6  $\mu\text{mol}$ ) in anhydrous DCM (3.0 mL). The reaction was stirred under an atmosphere of  $\text{N}_2$  at room temperature for 16h. The solution was concentrated *in vacuo*, the resulting residue dissolved in diethyl ether (10.0 mL) and washed with water (2 x 10.0mL). The organic layer was separated and washed with a saturated solution of  $\text{NaHCO}_3$  (2 x 10.0mL) and brine (10.0mL), dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a yellow oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 8:2, as the eluent but no product was isolated.

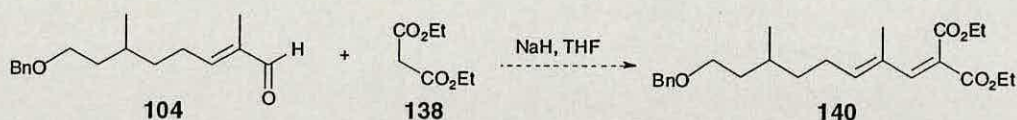
**Diethyl 12-benzyloxy-2-carbethoxy-6,10-dimethyldodec-2,4,6-trienoate, 141.**



A stirred solution of 8-benzyloxy-2,6-dimethyl-2-octenal, **104**, (100.0 mg, 384.0  $\mu\text{mol}$ ) and diethyl ethylidene malonate, **132**, (78.7 mg, 422.0  $\mu\text{mol}$ ) in anhydrous DCM (5.0 mL) was treated with a solution of acetic acid (8.2 mg, 153.6  $\mu\text{mol}$ ) and piperidine (13.0 mg, 153.6  $\mu\text{mol}$ ) in anhydrous DCM (3.0 mL). The reaction was stirred under an atmosphere of  $\text{N}_2$  at room temperature for 16h. The solution was concentrated *in vacuo*, the resulting residue dissolved in diethyl ether (10.0 mL) and washed with water (2 x 10.0 mL). The organic layer was separated and washed with a saturated solution of  $\text{NaHCO}_3$  (2 x 10.0mL) and brine (10.0 mL), dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a yellow oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 8:2, as the eluent but no product was isolated.

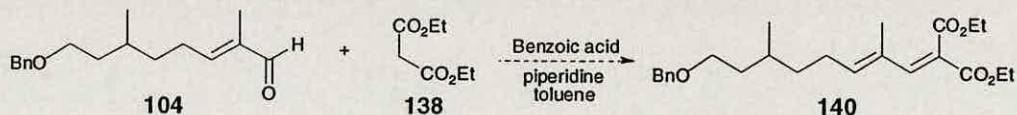


**Diethyl 10-benzyloxy-2-carbethoxy-4,8-dimethyldec-2,4-dienoate, 140.**



A stirred solution of diethyl malonate, **138**, (67.6 mg, 422.0  $\mu$ mol) in anhydrous THF (3.0 mL) was cooled to 0°C and treated with a 60% dispersion of sodium hydride in mineral oil (10.1 mg, 422.0  $\mu$ mol). The reaction mixture was stirred at 0°C for 45 min under an atmosphere of N<sub>2</sub> before the addition of a solution of 8-benzyloxy-2,6-dimethyl-2-octenal, **104**, (100.0 mg, 384.0  $\mu$ mol) in anhydrous THF (3.0 mL). The reaction mixture was gradually allowed to warm to room temperature and stirred under an atmosphere of N<sub>2</sub> for 16h. The solution was diluted with water (5.0 mL) and extracted with ethyl acetate (3 x 10.0 mL), the organic layers were combined, washed with brine (15.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a yellow oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent but no product was isolated.

**Diethyl 10-benzyloxy-2-carbethoxy-4,8-dimethyldec-2,4-dienoate, 140.**

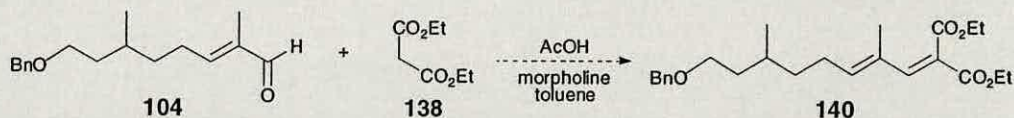


A stirred solution of 8-benzyloxy-2,6-dimethyl-2-octenal, **104**, (100.0 mg, 384.0  $\mu$ mol) and diethyl malonate, **138**, (29.0  $\mu$ L, 188.0  $\mu$ mol) in anhydrous toluene (5.0 mL) was treated with a solution of benzoic acid (0.9 mg, 7.7  $\mu$ mol) and piperidine (11.0  $\mu$ L, 11.5  $\mu$ mol) in anhydrous toluene (1.0 mL). The reaction was heated at reflux under an atmosphere of N<sub>2</sub> in a Dean-Stark apparatus for 16h. The solution was allowed to cool to room temperature and diluted with toluene. The organic layer was washed with water (2 x 10.0 mL), a saturated solution of NaHCO<sub>3</sub> (2 x 10.0 mL) and brine (10.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a brown oil. The crude mixture was separated by column



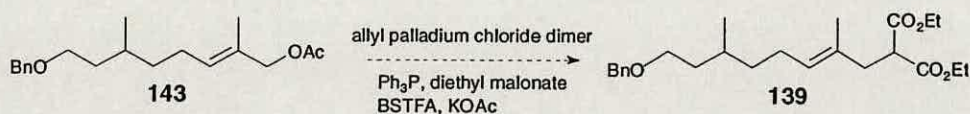
chromatography on silica using petroleum ether:ethyl acetate, 8:2, as the eluent but no product was isolated.

**Diethyl 10-benzyloxy-2-carbethoxy-4,8-dimethyldec-2,4-dienoate, 140.**



A stirred solution of 8-benzyloxy-2,6-dimethyl-2-octenal, **104**, (100.0 mg, 384.0  $\mu\text{mol}$ ) and diethyl malonate, **138**, (52.4  $\mu\text{L}$ , 345.0  $\mu\text{mol}$ ) in anhydrous toluene (5.0 mL) was treated with a solution of acetic acid (4.2  $\mu\text{L}$ , 69.0  $\mu\text{mol}$ ) and morpholine (1.2  $\mu\text{L}$ , 13.8  $\mu\text{mol}$ ) in anhydrous toluene (1.0 mL). The reaction was heated at reflux under an atmosphere of  $\text{N}_2$  in a Dean-Stark apparatus for 16h. The solution was allowed to cool to room temperature and diluted with toluene. The organic layer was washed with water (2 x 10.0 mL), a saturated solution of  $\text{NaHCO}_3$  (2 x 10.0 mL) and brine (10.0 mL), dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a dark yellow oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent but no product was isolated.

**Diethyl 2-(8-benzyloxy-2,6-dimethyloct-2-en-1-yl) malonate, 139.**



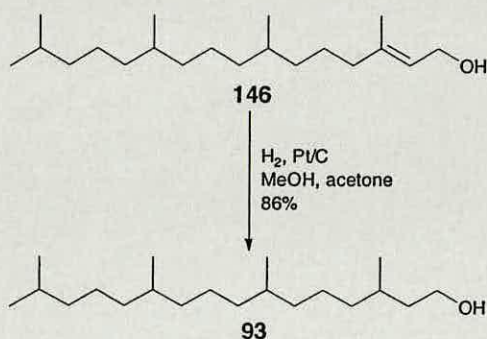
A solution of allyl palladium chloride dimer (0.8 mg, 2.17  $\mu\text{mol}$ ) and triphenylphosphine (1.4 mg, 5.43  $\mu\text{mol}$ ) in anhydrous DCM (2.0 mL) was stirred at room temperature under an atmosphere of  $\text{N}_2$  for 1h. A solution of 8-benzyloxy-2,6-dimethyloct-2-en-1-yl acetate (66.1 mg, 217.0  $\mu\text{mol}$ ) in anhydrous DCM (2.0 mL) was added and the reaction mixture was cooled to  $-20^\circ\text{C}$  before the addition of diethyl malonate, **138**, (98.9  $\mu\text{L}$ , 652.0  $\mu\text{mol}$ ), BSTFA (173.2  $\mu\text{L}$ , 652.0  $\mu\text{mol}$ ) and potassium acetate (0.2 mg, 2.17  $\mu\text{mol}$ ). The reaction was stirred at  $-20^\circ\text{C}$  for 10h and then quenched by the addition of a saturated solution of ammonium chloride (6.0



mL). The organic layer was separated, washed with water (2 x 10.0 mL), a saturated solution of  $\text{NaHCO}_3$  (2 x 10.0 mL) and brine (10.0 mL), dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a dark yellow oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent but no product was isolated.

### 4.3. Synthesis of lipid-linked monosaccharide and disaccharide.

#### 3,7,11,15-Tetramethylhexadecan-1-ol (Phytanol), **93**.<sup>35</sup>

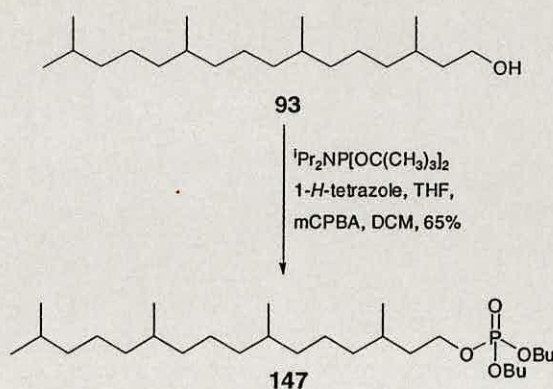


A stirred solution of 3,7,11,15-tetramethylhexadec-2-en-1-ol, **146**, (2.0 g, 6.7 mmol) in anhydrous methanol (20.0 mL) was treated with finely divided 5% platinum on charcoal (288.0 mg) as catalyst and placed under an atmosphere of N<sub>2</sub>. The system was evacuated and flushed with hydrogen. This process was repeated twice and the reaction mixture was then allowed to stir under an atmosphere of hydrogen for 3.5h. The solution was filtered through a pad of celite, which was washed well with methanol (3 x 20.0mL). The combined filtrate and washings were concentrated *in vacuo* to give a pale yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent to afford the product, **93**, (1.7 g, 86%) as a clear oil.

R<sub>f</sub> (petroleum ether:ethyl acetate, 6:4) 0.47; HRMS (FAB) 297.3153 ([M]<sup>+</sup>, C<sub>20</sub>H<sub>41</sub>O requires 297.3157); LRMS (FAB) 297 (C<sub>20</sub>H<sub>41</sub>O); δ<sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 3.96-3.83 (2H, m, CH<sub>2</sub>OH), 1.86-1.67 (4H, m, 2 x CH<sub>2</sub>), 1.63-1.24 (21H, m, 8 x CH<sub>2</sub>, 4 x CH, OH), 1.11-1.03 (15H, m, 5 x CH<sub>3</sub>); δ<sub>C</sub> (CDCl<sub>3</sub>, 63MHz) 61.4 (CH<sub>2</sub>OH), 40.2 and 40.1 (CH<sub>2</sub>), 39.5 (2 x CH<sub>2</sub>), 37.6, 37.5 and 37.4 (CH<sub>2</sub>), 32.9 (2 x CH), 29.7 and 28.1 (CH), 24.9, 24.6 and 24.5 (CH<sub>2</sub>), 22.9 (2 x CH<sub>3</sub>), 22.8, 19.9 and 19.8 (CH<sub>3</sub>); ν<sub>max</sub> (film)/cm<sup>-1</sup> 3615 (OH), 2927, 2869 (CH).



**Di-*tert*-butyl 3,7,11,15-Tetramethylhexadecyl phosphate (Di-*tert*-butyl phytanyl phosphate), 147.**<sup>35</sup>



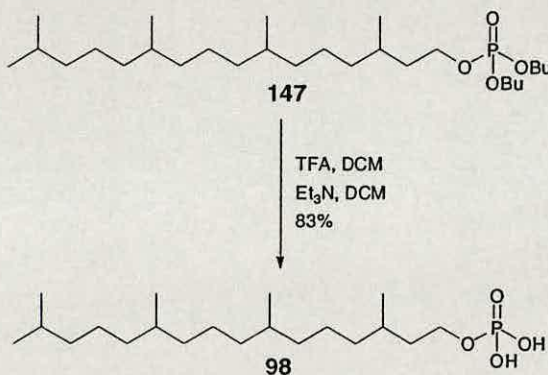
A stirred solution of 3,7,11,15-tetramethylhexadecan-1-ol, **93**, (1.6 g, 5.4 mmol) in anhydrous THF (20.0 mL) was treated with *N,N'*-di-*tert*-butyl diisopropylphosphoramidite (3.8 mL, 12.1 mmol) and 1-*H*-tetrazole (1.2 g, 17.2 mmol). A white precipitate developed and the resulting solution was stirred at room temperature for 3h. The reaction mixture was cooled to 0°C in an ice bath and treated with a solution of mCPBA (3.3 g, 19.3 mmol) in anhydrous DCM (20.0 mL). The reaction mixture was gradually allowed to warm to room temperature and stirred under N<sub>2</sub> for 1h. The clear, pale blue reaction mixture was treated with a saturated solution of sodium sulfite (30.0 mL) and stirred for 30min under N<sub>2</sub>. The solution was diluted with DCM (30.0 mL), the aqueous layer separated and extracted with DCM (2 x 15.0mL). The organic extracts were combined, washed with a saturated solution of NaHCO<sub>3</sub> (2 x 30.0mL) and brine (30.0mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 8:2, as the eluent to afford the product, **147**, (1.7 g, 65%) as a clear oil.

R<sub>f</sub> (petroleum ether:ethyl acetate, 8:2) 0.30; HRMS (FAB) 491.4226 ([M+H]<sup>+</sup>, C<sub>28</sub>H<sub>60</sub>O<sub>4</sub>P requires 491.4229); LRMS (FAB) 491 (C<sub>28</sub>H<sub>60</sub>O<sub>4</sub>P), 435 (C<sub>24</sub>H<sub>52</sub>O<sub>4</sub>P), 379 (C<sub>20</sub>H<sub>44</sub>O<sub>4</sub>P); δ<sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 4.11-4.02 (2H, m, CH<sub>2</sub>OP), 1.85-1.65 (4H, m, 2 x CH<sub>2</sub>), 1.55 (18H, s, 2 x C(CH<sub>3</sub>)<sub>3</sub>), 1.36-1.27 (20H, br m, 4 x CH, 8 x CH<sub>2</sub>), 0.99-0.90 (15H, m, 5 x CH<sub>3</sub>); δ<sub>C</sub> (CDCl<sub>3</sub>, 63MHz) 81.8 and 81.7 (C(CH<sub>3</sub>)<sub>3</sub>), 65.1, 65.0, 60.3 and 39.2 (CH<sub>2</sub>), 37.2 (2 x CH<sub>2</sub>), 37.1 (CH<sub>2</sub>), 32.6 (CH), 29.8 (2 x CH),



29.1 (CH), 27.8, 24.6, 24.3 and 24.2 (CH<sub>2</sub>), 22.6 (3 x CH<sub>3</sub>), 22.5, 21.8 and 20.9 (CH<sub>3</sub>), 19.6 (3 x CH<sub>3</sub>), 19.3 and 14.1 (CH<sub>3</sub>);  $\delta_P$  (CDCl<sub>3</sub>, 101MHz) -8.4 (s, P=O);  $\nu_{\max}$  (film)/cm<sup>-1</sup> 2983, 2956, 2928 (CH), 1172 (P=O), 1039 (P-O-C).

### 3,7,11,15-Tetramethylhexadecyl phosphate (Phytanyl phosphate), **98**.<sup>35</sup>

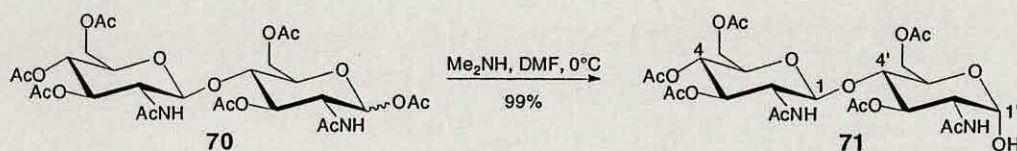


A stirred solution of di-*tert*-butyl 3,7,11,15-tetramethylhexadecyl phosphate, **147**, (0.7 g, 1.4 mmol) in anhydrous DCM (5.0 mL) was treated with TFA (2.1 mL, 27.7 mmol) and stirred under N<sub>2</sub> for 30 min. The reaction mixture was concentrated *in vacuo* to yield a colourless oil. This residue was dissolved in a 2:1 mixture of anhydrous DCM : MeOH (9.0 mL) and treated with triethylamine (3.0 mL, 27.7 mmol). The reaction mixture was stirred for 10 min under N<sub>2</sub> then concentrated *in vacuo* and azeotroped with anhydrous toluene (3 x 30.0 mL) to afford the product, **98**, (0.4 g, 83%) as a colourless oil.

R<sub>f</sub> (DCM:MeOH:H<sub>2</sub>O:2M aq NH<sub>4</sub>HCO<sub>3</sub>, 6.5:3.5:0.4:0.4) 0.76; HRMS (FAB) 401.2800 ([M+Na]<sup>+</sup>, C<sub>20</sub>H<sub>43</sub>O<sub>4</sub>PNa requires 401.2797); LRMS (FAB) 410 (C<sub>20</sub>H<sub>43</sub>O<sub>4</sub>PNa);  $\delta_H$  (CDCl<sub>3</sub>, 250MHz) 3.94-3.82 (2H, m, CH<sub>2</sub>OP), 1.61-1.39 (4H, m, 2 x CH<sub>2</sub>), 1.31-0.97 (20H, br m, 4 x CH, 8 x CH<sub>2</sub>), 0.81-0.75 (15H, m, 5 x CH<sub>3</sub>);  $\delta_C$  (CDCl<sub>3</sub>, 63MHz) 63.8 (CH<sub>2</sub>OP), 45.4 (2 x CH<sub>2</sub>), 39.2 (2 x CH<sub>2</sub>), 37.4 and 37.3 (CH<sub>2</sub>), 37.2 (2 x CH<sub>2</sub>), 32.7 (2 x CH), 29.3 and 27.8 (CH), 24.7 and 24.4 (CH<sub>2</sub>), 22.5, 19.5, 19.3, 19.2 and 18.9 (CH<sub>3</sub>);  $\delta_P$  (CDCl<sub>3</sub>, 101MHz) 3.0 (s, P=O);  $\nu_{\max}$  (film)/cm<sup>-1</sup> 2986, 2956, 2928 (CH), 1152 (P=O), 1013 (P-OH).



**2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl-(1-4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranose (chitobiose heptaacetate), **70**.<sup>82</sup>**

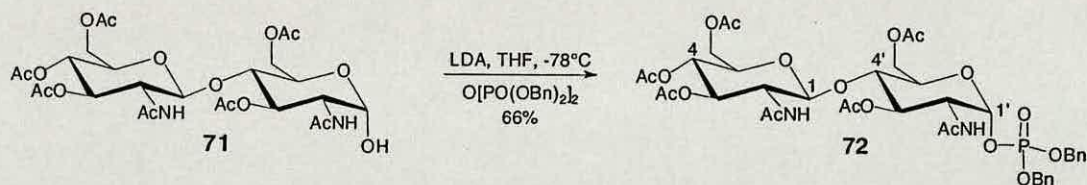


A stirred solution of chitobiose octaacetate, **70**, (1.0 g, 1.5 mmol) in anhydrous DMF (10.0 mL) was cooled to 0°C under Ar and treated with a 2 M solution of dimethylamine (10.0 mL, 20.0 mmol) in THF. The reaction mixture was stirred at 0°C for 1h under Ar and then allowed to gradually warm to room temperature. A second aliquot of the 2 M dimethylamine solution (10.0 mL, 20.0 mmol) was added and the reaction was stirred at room temperature under Ar for 16h. The reaction mixture was concentrated *in vacuo* to yield a yellow solid which was crystallised from DCM and petroleum ether to give the product, **71**, (0.9 g, 99%) as a white solid.

$R_f$  (DCM:MeOH, 9:1) 0.27; Mp 240.1-242.5 °C (Lit 245.0-247.0 °C<sup>82</sup>);  $[\alpha]_D -23.4^\circ$  ( $c$  1.0, CHCl<sub>3</sub>) (Lit  $[\alpha]_D -30.0^\circ$  ( $c$  1.0, CHCl<sub>3</sub>)<sup>82</sup>); HRMS (FAB) 635.2299 ( $[M+H]^+$ , C<sub>26</sub>H<sub>39</sub>O<sub>16</sub>N<sub>2</sub> requires 635.2299);  $\delta_H$  (CDCl<sub>3</sub>, 250MHz) 5.54 (1H, dd,  $J_{3',2'}$  10.3 Hz,  $J_{3',4'}$  9.5 Hz, H3'), 5.18 (1H, d,  $J_{1',2'}$  3.6 Hz, H1'), 5.10 (1H, dd,  $J_{3,2}$  10.1 Hz,  $J_{3,4}$  9.0 Hz, H3), 4.59 (1H, d,  $J_{1,2}$  8.3 Hz, H1), 4.48 (1H, dd,  $J_{H6a-H6b}$  12.6 Hz,  $J_{H6b-H5}$  4.1 Hz, H6b), 4.43 (1H, dd,  $J_{H6a'-H6b'}$  12.2 Hz,  $J_{H6a'-H5'}$  3.8 Hz, H6a'), 4.35-4.28 (1H, m, H5), 4.26 (1H, dd,  $J_{H6b-H6a}$  12.6 Hz,  $J_{H6a-H5}$  3.2 Hz, H6a), 4.19 (1H, dd,  $J_{2',1'}$  3.6 Hz,  $J_{2',3'}$  10.3 Hz, H2'), 4.07 (1H, dd,  $J_{4,3}$  9.0 Hz,  $J_{4,5}$  9.9 Hz, H4), 4.05-3.99 (1H, m, H5'), 3.96 (1H, dd,  $J_{2,1}$  8.3 Hz,  $J_{2,3}$  10.1 Hz, H2), 3.74 (1H, dd,  $J_{H6a'-H6b'}$  12.2 Hz,  $J_{H6b'-H5'}$  9.4 Hz, H6b'), 3.70-3.64 (1H, m, H4'), 2.15-1.93 (21H, m, 7 x COCH<sub>3</sub>);  $\delta_C$  (CDCl<sub>3</sub>, 63MHz) 172.5, 172.4, 171.9, 171.7, 171.6, 171.1 and 169.6 (CO), 101.9, 91.4, 76.3, 72.1, 71.6, 71.0, 68.2 and 68.0 (CH), 63.0 and 61.7 (CH<sub>2</sub>), 53.8 and 51.9 (CH), 23.9 (CH<sub>3</sub>), 22.8 (2 x CH<sub>3</sub>), 20.9 (3 x CH<sub>3</sub>), 20.7 (CH<sub>3</sub>);  $\nu_{max}$  (film)/cm<sup>-1</sup> 3374 (OH), 2986 (CH), 1742 (OC=O), 1674 (N-C=O) and 1092 (C-O-C).



**2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl-(1-4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate dibenzyl ester, **72**.<sup>82</sup>**



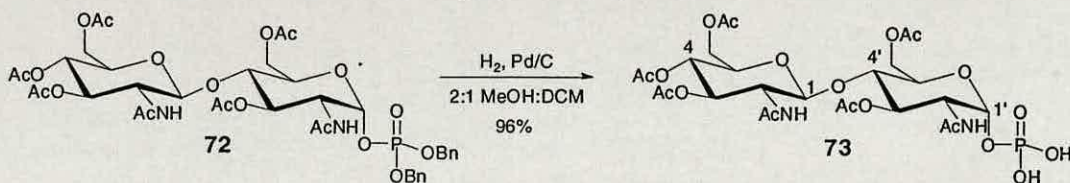
A stirred solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl-(1-4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranose, **71**, (349.5 mg, 0.6 mmol) in anhydrous THF (5.0 mL) was cooled to  $-78^{\circ}\text{C}$  and treated with a 1.8 M solution of LDA in 1:1:1 heptane/THF/ethylbenzene (0.4 mL, 0.8 mmol). The reaction mixture was stirred under Ar for 45 min whereupon a solution of tetrabenzyl pyrophosphate (0.4 g, 0.8 mmol) in anhydrous THF (5.0 mL) was added. The solution was stirred at  $-78^{\circ}\text{C}$  for 4h under Ar after which time it was gradually allowed to warm to room temperature. The reaction was concentrated *in vacuo* to yield a white residue. This was purified by column chromatography on silica using DCM:MeOH, 24:1, as the eluent to afford the product, **72**, (322.8 mg, 66%) as a white solid.

$R_f$  (DCM:MeOH, 9:1) 0.59; Mp  $146.2\text{--}148.7^{\circ}\text{C}$  (Lit  $139.0\text{--}141.0^{\circ}\text{C}$ <sup>38</sup>);  $[\alpha]_D -11.0^{\circ}$  ( $c$  1.4,  $\text{CHCl}_3$ ) (Lit  $[\alpha]_D -15.0^{\circ}$  ( $c$  1.1,  $\text{CHCl}_3$ )<sup>82</sup>); HRMS (FAB) 895.2906 ( $[\text{M}+\text{H}]^+$ ,  $\text{C}_{40}\text{H}_{52}\text{O}_{19}\text{N}_2\text{P}$  requires 895.2902); CHN found %C 53.59, %H 5.68, %N 3.09 (required %C 53.69, %H 5.71, %N 3.13);  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 250MHz) 7.32-7.24 (10H, m, ArH), 5.83 (1H, dd,  $J_{3',4'}$  9.6 Hz,  $J_{3',2'}$  9.3 Hz, H3'), 5.51 (1H, dd,  $J_{\text{H1}',\text{P}}$  5.9 Hz,  $J_{1',2'}$  3.4 Hz, H1'), 5.07 (1H, dd,  $J_{2,3}$  10.3 Hz,  $J_{3,4}$  9.0 Hz, H3), 4.99-4.87 (5H, m, H4, 2 x  $\text{POCH}_2$ ), 4.44 (1H, d,  $J_{1,2}$  8.3 Hz, H1), 4.27 (1H, dd,  $J_{\text{H6a-H6b}}$  12.7 Hz,  $J_{\text{H6b-H5}}$  4.3 Hz, H6b), 4.18 (1H, dd,  $J_{\text{H6a'-H6b'}}$  12.1 Hz,  $J_{\text{H6a'-H5'}}$  2.9 Hz, H6a'), 4.11 (1H, dd,  $J_{2',1'}$  3.4 Hz,  $J_{2',3'}$  9.3 Hz, H2'), 3.94 (1H, dd,  $J_{\text{H6a-H6b}}$  12.7 Hz,  $J_{\text{H6a-H5}}$  2.2 Hz, H6a), 3.83-3.79 (1H, m, H5'), 3.73 (1H, dd,  $J_{2,1}$  8.3 Hz,  $J_{2,3}$  10.3 Hz, H2), 3.62 (1H, d,  $J_{4',5'}$  9.8 Hz,  $J_{3',4'}$  9.6 Hz, H4'), 3.54-3.50 (1H, m, H5), 3.49-3.45 (1H, m, H6b'), 1.96-1.86 (15H, m, 5 x  $\text{COCH}_3$ ), 1.82 and 1.58 (2 x 3H, 2 x s, 2 x  $\text{COCH}_3$ );  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 63MHz) 170.9, 170.8, 170.6, 170.4 (CO), 170.2 (2 x CO), 169.2 (CO), 135.1 and 135.0 (ArC),



128.8 (2 x ArCH), 128.7 (2 x ArCH), 128.6 (2 x ArCH), 128.0 (2 x ArCH), 127.9 (2 x ArCH), 101.1, 95.9, 75.5, 72.3, 71.8, 70.5 and 70.0 (CH), 69.9 and 69.8 (CH<sub>2</sub>), 67.8 (CH), 61.5 and 61.1 (CH<sub>2</sub>), 54.7 and 51.7 (CH), 23.1, 22.6, 20.8 (CH<sub>3</sub>), 20.6 (2 x CH<sub>3</sub>), 20.5 (2 x CH<sub>3</sub>);  $\delta_P$  (CDCl<sub>3</sub>, 101MHz) 0.0 (s, P=O);  $\nu_{\max}$  (film)/cm<sup>-1</sup> 2986 (CH), 1744 (OC=O), 1604 (N-C=O) and 1156 (P-O-C).

**2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl-(1-4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate, **73**.<sup>82</sup>**



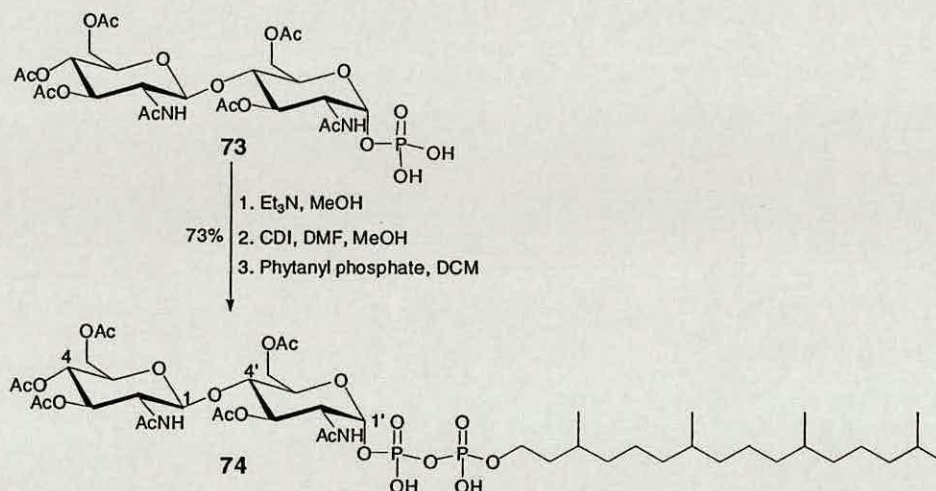
A stirred solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl-(1-4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate dibenzyl ester, **72**, (469.0 mg, 0.5 mmol) in a 2:1 mixture of anhydrous MeOH:DCM (15.0 mL) was treated with finely divided 10% palladium on charcoal (168.0 mg) as catalyst and placed under an atmosphere of N<sub>2</sub>. The system was evacuated and flushed with hydrogen. This process was repeated twice and the reaction mixture was then allowed to stir under an atmosphere of hydrogen for 3.5h. The solution was filtered through a pad of celite, which was washed well with MeOH (3 x 20.0 mL). The combined filtrate and washings were concentrated *in vacuo* to give a pale yellow solid. This was azeotroped with anhydrous toluene (3 x 15.0 mL) and dried under high vacuum to afford the product, **73**, (369.8 mg, 96%) as a white solid.

$R_f$  (DCM:MeOH, 9:1) 0.29; Mp 184.5-186.7 °C;  $[\alpha]_D^{25}$  22.4° (*c* 1.0, CHCl<sub>3</sub>); HRMS (FAB) 737.1781 ([M+Na]<sup>+</sup>, C<sub>26</sub>H<sub>39</sub>O<sub>19</sub>N<sub>2</sub>PNa requires 737.1783);  $\delta_H$  (CDCl<sub>3</sub>, 250MHz) 5.39 (2H, br s, P(OH)<sub>2</sub>), 5.15 (1H, dd,  $J_{3,2}$  9.8 Hz,  $J_{3,4}$  9.3 Hz, H3), 5.05 (1H, dd,  $J_{3',2'}$  10.5 Hz,  $J_{3',4'}$  9.3 Hz, H3'), 4.96 (1H, dd,  $J_{H1',P}$  10.8 Hz,  $J_{1',2'}$  3.8 Hz, H1'), 4.90 (1H, dd,  $J_{3,4}$  9.3 Hz,  $J_{4,5}$  9.7 Hz, H4), 4.55-4.50 (1H, m, H6b'), 4.44 (1H, d,  $J_{1,2}$  8.4 Hz, H1), 4.30 (1H, dd,  $J_{H6a-H6b}$  12.3 Hz,  $J_{H6b-H5}$  3.8 Hz, H6b), 4.26 (1H, dd,  $J_{3',4'}$  9.3 Hz,  $J_{4',5'}$  9.9 Hz, H4'), 4.17-4.10 (1H, br m, H6a'), 4.01 (1H, dd,  $J_{2',3'}$  10.5



Hz,  $J_{2',1'}$  3.8 Hz, H2'), 3.91 (1H, dd,  $J_{H6a-H6b}$  12.3 Hz,  $J_{H6a-H5}$  2.1 Hz, H6a), 3.83 (1H, dd,  $J_{2,3}$  9.8 Hz,  $J_{2,1}$  8.4 Hz, H2), 3.54-3.50 (1H, m, H5), 3.39-3.26 (1H, m, H5'), 2.02, 1.97, 1.94, 1.93, 1.92, 1.90 and 1.80 (7 x 3H, 7 x s, 7 x COCH<sub>3</sub>);  $\delta_C$  (CDCl<sub>3</sub>, 63MHz) 177.5, 177.1, 176.6, 176.4 and 176.2 (CO), 175.3 (2 x CO), 106.1, 80.8, 77.9, 77.6, 76.9, 76.2, 75.1 and 73.8 (CH), 67.2 (CH<sub>2</sub>), 60.2 and 57.1 (CH), 51.6 (CH<sub>2</sub>), 27.9 (2 x CH<sub>3</sub>), 27.7 (2 x CH<sub>3</sub>), 25.8 (3 x CH<sub>3</sub>);  $\delta_P$  (CDCl<sub>3</sub>, 101MHz) 0.0 (s, P=O);  $\nu_{max}$  (film)/cm<sup>-1</sup> 2986 (CH), 1712 (OC=O), 1603 (N-C=O), 1044 (P-O-C) and 986 (P-OH).

***P*<sub>1</sub>-Phytanyl-*P*<sub>2</sub>-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl-(1-4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl]-pyrophosphate, 74.<sup>82</sup>**



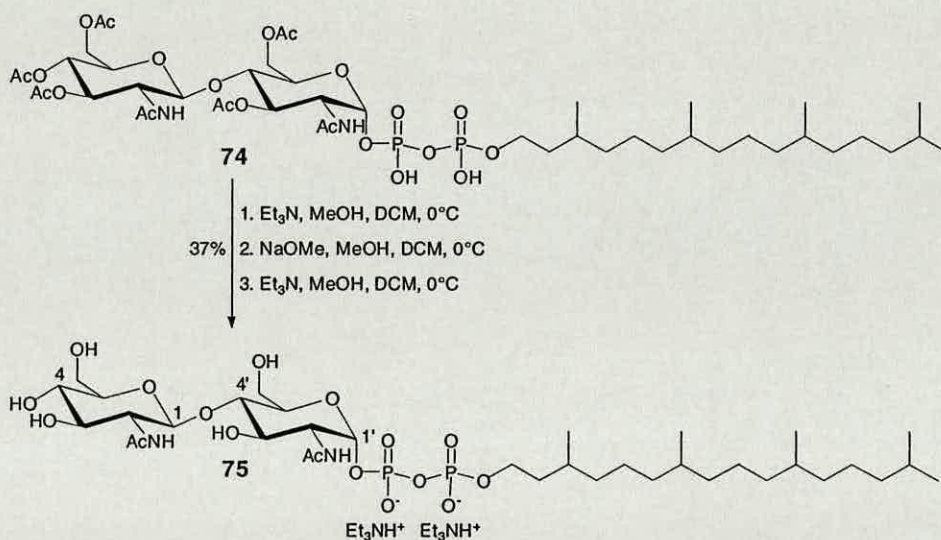
A stirred solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl-(1-4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate, **73**, (150.0 mg, 0.2 mmol) in anhydrous methanol (4.0 mL) was treated with triethylamine (0.8 mL, 0.6 mmol). The reaction mixture was stirred under N<sub>2</sub> at room temperature for 5 min, then concentrated and evaporated with anhydrous toluene (3 x 3.0 mL). The resulting pale yellow residue was dissolved in anhydrous DMF (3.0 mL) and treated with a solution of *N,N'*-carbonyldiimidazole (188.0 mg, 1.2 mmol) in anhydrous DMF (3.0 mL). The reaction mixture was stirred under N<sub>2</sub> at room temperature for 4.5h. Anhydrous methanol (80.0  $\mu$ L) was added to the reaction, which was stirred



under N<sub>2</sub> at room temperature for 30 min. The reaction mixture was then treated with a solution of phytanyl phosphate, **98**, (142.0 mg, 0.3 mmol) in anhydrous DCM (2.0 mL) and the reaction mixture was stirred under N<sub>2</sub> at room temperature for 3.5 days after which time the solution was concentrated *in vacuo* to yield a yellow residue. This was purified by column chromatography on silica using DCM:MeOH:H<sub>2</sub>O, 8:2:0.4, as eluent to afford the product, **74**, (165.8 mg, 73%) as a white solid.

R<sub>f</sub> (DCM:MeOH:H<sub>2</sub>O:2M aq NH<sub>4</sub>HCO<sub>3</sub>, 6.5:3.5:0.4:0.4) 0.53; HRMS (FAB) 1097.4560 ([M+Na]<sup>+</sup>, C<sub>46</sub>H<sub>80</sub>O<sub>22</sub>N<sub>2</sub>P<sub>2</sub>Na requires 1097.4576); δ<sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 5.61-5.53 (1H, m, CH), 5.39-5.30 (2H, m, 2 x CH), 5.27-5.19 (1H, m, CH), 4.97-4.89 (1H, m, CH), 4.70-4.50 (1H, m, CH), 4.34-4.20 (2H, m, 2 x CH), 3.99-3.82 (4H, m, POCH<sub>2</sub>, 2 x CH), 3.75-3.60 (2H, m, 2 x CH), 3.58-3.50 (2H, m, 2 x CH), 1.96-1.77 (21H, br m, 7 x COCH<sub>3</sub>), 1.44-1.38 (6H, m, 3 x CH<sub>2</sub>), 1.18-1.00 (18H, m, 4 x CH, 7 x CH<sub>2</sub>) and 0.79-0.70 (15H, m, 5 x CH<sub>3</sub>); δ<sub>P</sub> (CDCl<sub>3</sub>, 101MHz) -8.1 (s, P=O) and -11.5 (s, P=O); ν<sub>max</sub> (film)/cm<sup>-1</sup> 2986 (CH), 1744 (OC=O), 1604 (N-C=O) and 1156 (P-O-C).

***P*<sub>1</sub>-Phytanyl-*P*<sub>2</sub>-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1-4)-2-deoxy-α-D-glucopyranosyl]-pyrophosphate, **75**.<sup>82</sup>**

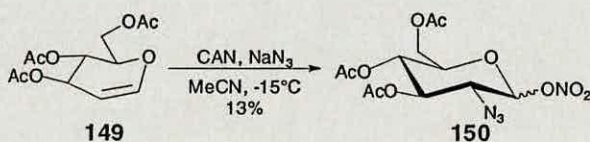


A stirred solution of *P*<sub>1</sub>-phytanyl-*P*<sub>2</sub>-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1-4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosyl]-pyrophosphate, **74**, (47.0 mg, 43.7 μmol) in anhydrous DCM (2.0 mL) and anhydrous methanol (1.0 mL) was cooled to 0°C and treated with triethylamine (0.6 mL). The reaction was stirred under N<sub>2</sub> for 5 min and then concentrated *in vacuo*. The resulting residue was azeotroped with anhydrous toluene (3 x 3.0 mL) and then re-dissolved in anhydrous DCM (2.0 mL) and anhydrous methanol (1.0 mL). The reaction was cooled to 0°C, treated with 0.5 M sodium methoxide solution (0.4 mL, 0.2 mmol) and stirred under N<sub>2</sub> for 15 min. The reaction mixture was passed through a H<sup>+</sup> Dowex AG50W-X8 ion exchange resin, which was then washed with anhydrous methanol (7 x 1.0 mL). The combined filtrate and washings were concentrated *in vacuo* and azeotroped with anhydrous toluene (3 x 3.0 mL). The resulting residue was dissolved in anhydrous DCM (2.0 mL) and anhydrous methanol (1.0 mL) and cooled to 0°C, treated with triethylamine (0.6 mL) and stirred under N<sub>2</sub> for 5 min. The solution was concentrated *in vacuo* and azeotroped with anhydrous toluene (3 x 3.0 mL) to afford the product, **75**, (14.1 mg, 37%) as a white solid.



$R_f$  (DCM:MeOH:H<sub>2</sub>O:2M aq NH<sub>4</sub>HCO<sub>3</sub>, 6.5:3.5:0.4:0.4) 0.27; HRMS (FAB) 887.4049 ([M+Na]<sup>+</sup>, C<sub>36</sub>H<sub>70</sub>O<sub>17</sub>N<sub>2</sub>P<sub>2</sub>Na requires 887.4048);  $\delta_H$  (CDCl<sub>3</sub>, trace MeOD, 250MHz) 11.61-11.55 (2H, m, 2 x CH), 11.47-11.38 (2H, m, 2 x CH), 8.11-8.06 (2H, m, 2 x CH), 7.95-7.57 (9H, br m, 5 x OH, 4 x CH), 7.27-7.22 (2H, m, 2 x CH), 5.99-5.82 (2H, m, 2 x CH), 5.63-5.52 (2H, m, CH<sub>2</sub>), 5.46-5.30 (2H, m, CH<sub>2</sub>), 5.22-5.06 (18H, br m, 4 x CH, 7 x CH<sub>2</sub>), 5.04-4.89 (4H, m, 2 x CH<sub>2</sub>), 4.81-4.68 (15H, m, 5 x CH<sub>3</sub>), 3.95 (6H, s, 2 x COCH<sub>3</sub>);  $\delta_P$  (CDCl<sub>3</sub>, 250MHz) -8.4 (s, P=O) and -11.8 (s, P=O);  $\nu_{max}$  (film)/cm<sup>-1</sup> 3279 (OH), 2986, 2961, 2927 and 2855 (CH), 1653 (N-C=O), 1096 (P-O-C) and 1014 (P-O-P).

## 2-Azido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl nitrate, **150**.<sup>130</sup>



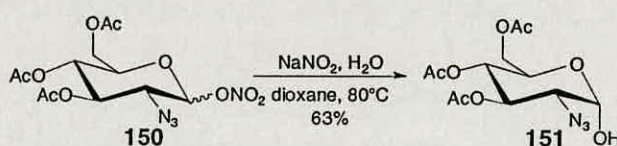
A stirred solution of tri-*O*-acetyl-D-glucal, **149**, (2.0 g, 7.4 mmol) in anhydrous acetonitrile (50.0 mL) was cooled to -15°C and treated with ceric ammonium nitrate (14.7 g, 26.8 mmol) and sodium azide (0.7 g, 11.0 mmol). The reaction mixture was stirred at -15°C for 6h and then at room temperature for 16h under an atmosphere of N<sub>2</sub>. The reaction mixture was poured onto ice-water (30.0 mL) and the resulting solution was extracted with diethyl ether (3 x 30.0 mL). The organic extracts were combined, washed with brine (50.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to give a yellow syrup. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent to afford the product, **150**, (3.5 g, 13%) as a clear oil.

$R_f$  (petroleum ether:ethyl acetate, 6:4) 0.65; HRMS (FAB) 377.0944 ([M+H]<sup>+</sup>, C<sub>12</sub>H<sub>17</sub>O<sub>10</sub>N<sub>4</sub> requires 377.0945); LRMS (FAB) 377 (C<sub>12</sub>H<sub>17</sub>O<sub>10</sub>N<sub>4</sub>), 314 (C<sub>12</sub>H<sub>16</sub>O<sub>7</sub>N<sub>3</sub>), 273 (C<sub>10</sub>H<sub>15</sub>O<sub>6</sub>N<sub>3</sub>);  $\delta_H$  (CDCl<sub>3</sub>, 250MHz) 6.23 (1H, d,  $J_{1,2}$  1.9 Hz, H1 $\alpha$ ), 5.63 (1H, d,  $J_{1,2}$  8.8 Hz, H1 $\beta$ ), 5.42 (1H, dd,  $J_{4,3}$  9.9 Hz,  $J_{4,5}$  9.7 Hz, H4 $\beta$ ), 5.34 (1H, dd,  $J_{H6a-H6b}$  13.3 Hz,  $J_{H6a-H5}$  2.8 Hz, H6a $\beta$ ), 5.28 (1H, dd,  $J_{H6b-H6a}$  13.3 Hz,



$J_{\text{H6b-H5}}$  3.9 Hz, H6b $\beta$ ), 5.18 (1H, dd,  $J_{3,2}$  9.9 Hz,  $J_{3,4}$  9.9 Hz, H3 $\beta$ ), 5.15 (1H, dd,  $J_{4,3}$  9.7 Hz,  $J_{4,5}$  9.4 Hz, H4 $\alpha$ ), 5.07 (1H, dd,  $J_{3,2}$  9.9 Hz,  $J_{3,4}$  9.7 Hz, H3 $\alpha$ ), 4.37 (1H, dd,  $J_{\text{H6b-H6a}}$  13.4 Hz,  $J_{\text{H6b-H5}}$  4.2 Hz, H6b $\alpha$ ), 4.29 (1H, dd,  $J_{\text{H6a-H6b}}$  13.4 Hz,  $J_{\text{H6a-H5}}$  2.1 Hz, H6a $\alpha$ ), 4.26-4.20 (2H, m, 2 x H5), 3.91-3.87 (1H, m, H2 $\alpha$ ), 3.71 (1H, dd,  $J_{1,2}$  8.8 Hz,  $J_{2,3}$  9.9 Hz, H2 $\beta$ ), 2.14, 2.11, 2.09, 2.07, 2.06 and 2.05 (6 x 3H, 6 x s, 6 x COCH<sub>3</sub>);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>, 63MHz) 170.9, 170.8, 170.2, 170.1, 169.9 and 169.7 (CO), 98.1, 97.5, 96.7, 95.5, 73.3, 71.6, 70.8, 67.9 and 65.2 (CH), 61.8 and 61.6 (CH<sub>2</sub>), 59.2 (CH), 21.4, 21.1, 21.0, 20.9, 20.8 and 20.7 (CH<sub>3</sub>).

## 2-Azido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranose, **151**.<sup>132</sup>



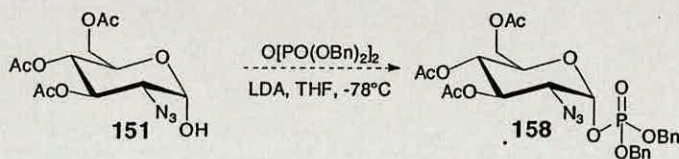
A stirred solution of 2-azido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl nitrate, **150**, (0.6 g, 1.6 mmol) in dioxane (10.0 mL) was treated with a solution of sodium nitrite (0.33 g, 4.8 mmol) in water (3.0 mL). The reaction mixture was stirred at 80°C for 6h and then poured onto ice-water (20.0 mL). The resulting solution was extracted with diethyl ether (3 x 15.0 mL). The organic extracts were combined, washed with a saturated solution of NaHCO<sub>3</sub> (20.0 mL) and brine (20.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to give a yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent to afford the product, **151**, (0.3 g, 63%) as a clear oil.

$R_f$  (petroleum ether:ethyl acetate, 6:4) 0.27; HRMS (FAB) 332.1104 ([M+H]<sup>+</sup>, C<sub>12</sub>H<sub>18</sub>O<sub>8</sub>N<sub>3</sub> requires 332.1094); LRMS (FAB) 354 (C<sub>12</sub>H<sub>17</sub>O<sub>8</sub>N<sub>3</sub>Na), 332 (C<sub>12</sub>H<sub>18</sub>O<sub>8</sub>N<sub>3</sub>), 306 (C<sub>12</sub>H<sub>19</sub>O<sub>8</sub>N), 43 (C<sub>2</sub>H<sub>3</sub>O);  $\delta_{\text{H}}$  (CDCl<sub>3</sub>, 250MHz) 5.51 (1H, dd,  $J_{3,2}$  10.0 Hz,  $J_{3,4}$  9.3 Hz, H3), 5.42 (1H, d,  $J_{1,2}$  3.7 Hz, H1), 5.37-5.30 (1H, m, H4), 5.24 (1H, br s, OH), 4.27 (1H, dd,  $J_{2,1}$  3.7 Hz,  $J_{2,3}$  10.0 Hz, H2), 4.18 (1H, dd,  $J_{\text{H6b-H6a}}$  12.7 Hz,  $J_{\text{H6b-H5}}$  4.1Hz, H6b), 4.04 (1H, dd,  $J_{\text{H6a-H6b}}$  12.7 Hz,  $J_{\text{H6a-H5}}$  1.8 Hz, H6a),



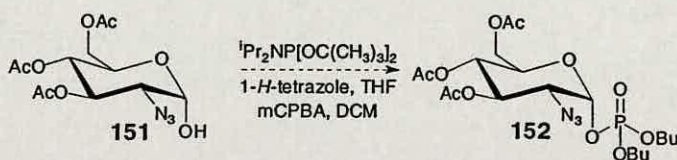
3.92-3.87 (1H, m, H5), 2.09, 2.06 and 2.02 (3 x 3H, 3 x s, 3 x COCH<sub>3</sub>);  $\delta_c$  (CDCl<sub>3</sub>, 63MHz) 170.9, 170.0 and 169.6 (CO), 91.9, 71.7, 70.3, 68.3 and 62.2 (CH), 61.7 (CH<sub>2</sub>), 20.9, 20.6 and 20.4 (CH<sub>3</sub>).

**2-Azido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate dibenzyl ester, 158.**



A stirred solution of 2-azido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranose, **151**, (40.3 mg, 121.0  $\mu\text{mol}$ ) in anhydrous THF (5.0 mL) was cooled to  $-78^\circ\text{C}$  and treated with a 1.8 M solution of LDA in 1:1:1 heptane/THF/ethylbenzene (85.6  $\mu\text{L}$ , 169.0  $\mu\text{mol}$ ). The reaction mixture was stirred under Ar at  $-78^\circ\text{C}$  for 45 min after which time a solution of tetrabenzylpyrophosphate (90.5 mg, 169.0  $\mu\text{mol}$ ) in anhydrous THF (2.0 mL) was added. The solution was stirred at  $-78^\circ\text{C}$  under Ar for a further 4h and then concentrated *in vacuo* to give a brown solid. The crude mixture was separated by column chromatography on silica using 4% MeOH in DCM as eluent but no product was isolated.

**Di-*tert*-butyl-2-azido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate, 152.**

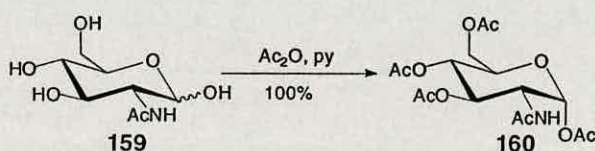


A stirred solution of 2-azido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranose, **151**, (79.0 mg, 240.0  $\mu\text{mol}$ ) in anhydrous DCM (5.0 mL) was treated with 1-*H*-tetrazole (0.1 g, 107.0  $\mu\text{mol}$ ) and *N,N'*-di-*tert*-butyl diethylphosphoramidite (169.3  $\mu\text{L}$ , 540.0  $\mu\text{mol}$ ). A white precipitate developed and the resulting solution was stirred at room temperature for 3h. The reaction mixture was cooled to  $0^\circ\text{C}$  in an ice bath and treated with a solution of mCPBA (0.2 g, 860.0  $\mu\text{mol}$ ) in anhydrous DCM (5.0 mL). The



reaction mixture was gradually allowed to warm to room temperature and stirred under N<sub>2</sub> for 1h. The clear, pale blue reaction mixture was treated with a saturated solution of sodium sulfite (15.0 mL) and stirred for 30 min under N<sub>2</sub>. The solution was diluted with DCM (15.0 mL), the aqueous layer separated and extracted with DCM (2 x 15.0 mL). The organic extracts were combined, washed with a saturated solution of NaHCO<sub>3</sub> (2 x 30.0 mL) and brine (30.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a yellow oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 8:2, as the eluent but no product was isolated.

**2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranose, **160**.<sup>103</sup>**



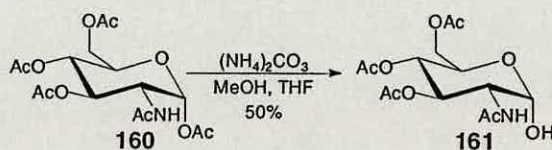
A stirred solution of *N*-acetyl-D-glucosamine, **159**, (25.0 g, 0.1 mol) in acetic anhydride (107.0 mL) was treated with pyridine (56.0 mL) and the reaction mixture was stirred under Ar at room temperature for 16h. The pale yellow solution was concentrated *in vacuo* to yield a clear syrup. This was azeotroped with toluene (3 x 40.0 mL) and dried under high vacuum to give the product, **160**, (44.2 g, 100%) as a white foam.

R<sub>f</sub> (6% MeOH in DCM) 0.36; Mp 127.0-129.3 °C (Lit. 136.0-137.0 °C<sup>133</sup>); [ $\alpha$ ]<sub>D</sub> 85.4° (*c* 1.2, CHCl<sub>3</sub>); HRMS (FAB) 390.1405 ([M+H]<sup>+</sup>, C<sub>16</sub>H<sub>24</sub>O<sub>10</sub>N requires 390.1400); LRMS (FAB) 412 (C<sub>16</sub>H<sub>23</sub>O<sub>10</sub>NNa), 390 (C<sub>16</sub>H<sub>24</sub>O<sub>10</sub>N), 330 (C<sub>14</sub>H<sub>20</sub>O<sub>8</sub>N), 43 (C<sub>2</sub>H<sub>3</sub>O); CHN found %C 49.33, %H 5.76, %N 3.34 (required %C 49.36, %H 5.91, %N 3.59);  $\delta$ <sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 6.29 (1H, d, *J*<sub>1,2</sub> 3.6 Hz, H1), 5.80 (1H, br d, *J* 9.1 Hz, NH), 5.37-5.32 (2H, m, H3, H4), 4.62-4.60 (1H, m, H2), 4.38 (1H, dd, *J*<sub>H6b-H6a</sub> 12.4 Hz, *J*<sub>H6b-H5</sub> 4.0 Hz, H6b), 4.20 (1H, d, *J*<sub>H6a-H5</sub> 2.3 Hz, H6a), 4.17-4.08 (1H, m, H5), 2.32, 2.21, 2.18, 2.16 and 2.06 (5 x 3H, 5 x s, 5 x COCH<sub>3</sub>);  $\delta$ <sub>C</sub> (CDCl<sub>3</sub>, 63MHz) 172.1, 171.2, 170.4, 169.5 and 169.1 (CO), 91.1, 71.7, 70.1 and 67.8 (CH), 61.6



(CH<sub>2</sub>), 51.4 (CH), 23.5, 21.4, 21.1, 20.9 and 20.7 (CH<sub>3</sub>);  $\nu_{\max}$  (film)/cm<sup>-1</sup> 2987 (CH), 1752 (OC=O), 1687 (N-C=O) and 1604 (NH).

**2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranose, **161**.<sup>103</sup>**

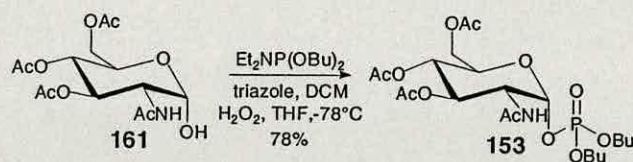


A stirred solution of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranose, **160**, (22.3 g, 57.3 mmol) in 2:1 anhydrous methanol:THF (180.0 mL) was treated with ammonium carbonate (22.6 g, 300.0 mmol). The reaction mixture was stirred under Ar at room temperature for 16h. The white solution was then concentrated *in vacuo* and the resulting yellow oil dissolved in DCM (50.0 mL). This solution was washed with water (3 x 40.0 mL) and brine (40.0 mL), dried over anhydrous MgSO<sub>4</sub> and concentrated *in vacuo* to yield the product, **161**, (10.1 g, 50%) as a white foam.

$R_f$  (6% MeOH in DCM) 0.25; Mp 52.2-53.9 °C (Lit. 84.0-85.0 °C<sup>143</sup>);  $[\alpha]_D$  51.2° (*c* 1.3, CHCl<sub>3</sub>) (Lit.  $[\alpha]_D$  52.2 ° (*c* 1.0, CHCl<sub>3</sub>)<sup>143</sup>); HRMS (FAB) 348.1294 ([M+H]<sup>+</sup>, C<sub>14</sub>H<sub>22</sub>O<sub>9</sub>N requires 348.1295); LRMS (FAB) 370 (C<sub>14</sub>H<sub>21</sub>O<sub>9</sub>NNa), 348 (C<sub>14</sub>H<sub>22</sub>O<sub>9</sub>N), 330 (C<sub>14</sub>H<sub>20</sub>O<sub>8</sub>N), 43 (C<sub>2</sub>H<sub>3</sub>O);  $\delta_H$  (CDCl<sub>3</sub>, 250MHz) 6.11 (1H, br d, *J* 9.4 Hz, NH), 5.39 (1H, dd, *J*<sub>3,4</sub> 9.5 Hz, *J*<sub>2,3</sub> 10.0 Hz, H3), 5.35-5.30 (1H, m, CH), 5.21 (1H, dd, *J*<sub>4,5</sub> 9.5 Hz, *J*<sub>4,3</sub> 9.5 Hz, H4), 4.75 (1H, br s, OH), 4.38 (1H, dd, *J*<sub>H6a-H6b</sub> 12.8 Hz, *J*<sub>H6a-H5</sub> 2.4 Hz, H6a), 4.30 (1H, dd, *J*<sub>3,2</sub> 10.0 Hz, *J*<sub>2,1</sub> 3.9 Hz, H2), 4.27-4.23 (1H, m, CH), 4.21 (1H, dd, *J*<sub>H6b-H5</sub> 3.7 Hz, *J*<sub>H6b-H6a</sub> 12.8 Hz, H6b), 2.19, 2.12, 2.10 and 2.05 (4 x 3H, 4 x s, 4 x COCH<sub>3</sub>);  $\delta_C$  (CDCl<sub>3</sub>, 63MHz) 171.9, 171.5, 171.0 and 169.9 (CO), 91.9, 71.4, 68.6 and 67.9 (CH), 62.5 (CH<sub>2</sub>), 52.7 (CH), 23.6 and 21.2 (CH<sub>3</sub>), 21.1 (2 x CH<sub>3</sub>);  $\nu_{\max}$  (film)/cm<sup>-1</sup> 3426 (OH), 2986 (CH), 1747 (OC=O) and 1683 (N-C=O).



**Di-*tert*-butyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate, 153.**

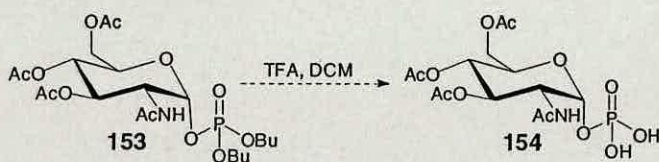


A stirred solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranose, **161**, (1.8 g, 5.2 mmol) in anhydrous DCM (20.0 mL) was treated with 1,2,4-triazole (1.4 g, 20.0 mmol) and *N,N'*-di-*tert*-butyl diethylphosphoramidite (4.3 mL, 20.0 mmol). The reaction mixture was stirred under an atmosphere of N<sub>2</sub> for 3h after which time it was cooled to  $-78^{\circ}\text{C}$  so that a 30% solution of hydrogen peroxide (13.0 mL) could be added in a dropwise manner. The reaction mixture was warmed to room temperature and stirred for a further 2h under N<sub>2</sub>. The reaction mixture was diluted with ice-cold diethyl ether (40.0 mL) and then carefully treated with a cold saturated solution of sodium thiosulphate (50.0 mL). The organic layer was separated, washed with a saturated solution of NaHCO<sub>3</sub> (2 x 40.0 mL) and brine (40.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered, then concentrated *in vacuo* to give a clear oil. This was purified by column chromatography on silica using 4% MeOH in DCM as the eluent to afford the product, **153**, (2.17 g, 78%) as a white foam.

R<sub>f</sub> (DCM:MeOH, 9:1) 0.31; HRMS (FAB) 540.2223 ([M+H]<sup>+</sup>, C<sub>22</sub>H<sub>39</sub>O<sub>12</sub>NP requires 540.2220); LRMS (FAB) 562 (C<sub>22</sub>H<sub>38</sub>O<sub>12</sub>NPNa), 540 (C<sub>22</sub>H<sub>39</sub>O<sub>12</sub>NP), 330 (C<sub>14</sub>H<sub>20</sub>O<sub>8</sub>N);  $\delta_{\text{H}}$  (CDCl<sub>3</sub>, 250MHz) 6.02 (1H, br d, *J* 9.2 Hz, NH), 5.54 (1H, dd, *J*<sub>H1-P</sub> 6.7 Hz, *J*<sub>1,2</sub> 3.3 Hz, H1), 5.23 (1H, dd, *J*<sub>3,2</sub> 9.5 Hz, *J*<sub>3,4</sub> 9.2 Hz, H3), 5.14 (1H, dd, *J*<sub>4,3</sub> 9.2 Hz, *J*<sub>4,5</sub> 9.3 Hz, H4), 4.41-4.30 (1H, m, H2), 4.19 (1H, dd, *J*<sub>H6b-H5</sub> 3.6 Hz, *J*<sub>H6b-H6a</sub> 12.2 Hz, H6b), 4.04 (1H, dd, *J*<sub>H6a-H6b</sub> 12.2 Hz, *J*<sub>H6a-H5</sub> 2.1 Hz, H6a), 3.75-3.68 (1H, m, H5), 1.47, 1.44, 1.43 and 1.42 (4 x 3H, 4 x s, 4 x COCH<sub>3</sub>), 1.38 (18H, s, 2 x C(CH<sub>3</sub>)<sub>3</sub>);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>, 63MHz) 171.4, 170.9, 170.2 and 169.4 (CO), 96.5 (CH), 84.5 and 84.3 (C(CH<sub>3</sub>)<sub>3</sub>), 73.0, 70.7 and 68.3 (CH), 62.2 (CH<sub>2</sub>), 54.2 (CH), 30.1 (3 x CH<sub>3</sub>), 29.9 (3 x CH<sub>3</sub>), 23.4 (CH<sub>3</sub>), 20.8 (2 x CH<sub>3</sub>), 13.6 (CH<sub>3</sub>);  $\delta_{\text{P}}$  (CDCl<sub>3</sub>, 101MHz)  $-12.6$  (s, P=O).



## 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate, 154.

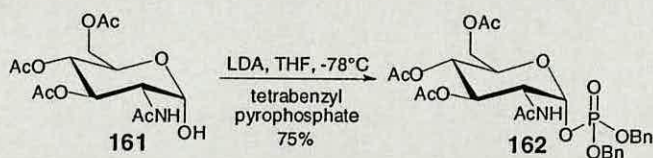


A stirred solution of di-*tert*-butyl-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate, **153**, (0.5 g, 0.9 mmol) in anhydrous DCM (2.0 mL) was treated with TFA (0.7 mL, 9.3 mmol) and stirred under N<sub>2</sub> for 10 min. The reaction mixture was concentrated *in vacuo* to yield a yellow oil. TLC and mass spectral analysis of the crude reaction mixture failed to produce any evidence of the desired product.

The reaction was repeated several times under different conditions as follows:

1. Compound **153** (0.5 g, 0.9 mmol) in anhydrous DCM (1.0 mL) was treated with citric acid (80.0 mg, 0.4 mmol) and stirred under N<sub>2</sub>. The reaction mixture was analysed by TLC at 30 min intervals but after 16h no product formation was observed;
2. Compound **153** (0.5 g, 0.9 mmol) in anhydrous DCM (1.0 mL) was treated with acetic acid (70.0  $\mu$ L, 0.9 mmol) and stirred under N<sub>2</sub>. The reaction mixture was analysed by TLC at 30 min intervals but after 16h no product formation was observed;
3. Compound **153** (0.5 g, 0.9 mmol) in anhydrous DCM (1.0 mL) was treated with TFA (7.0 mL, 9.0 mmol) and stirred under N<sub>2</sub>. The reaction mixture was analysed by TLC at 30 min intervals but after 16h no product formation was observed.

**2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate dibenzyl ester, **162**.<sup>144</sup>**

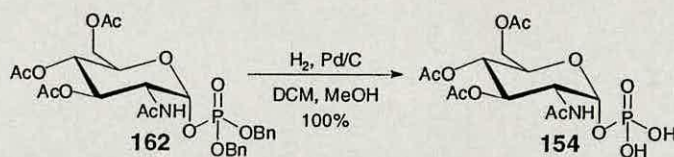


A stirred solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranose, **161**, (0.3 g, 0.7 mmol) in anhydrous THF (10.0 mL) was cooled to  $-78^{\circ}\text{C}$  and treated with a 1.8 M solution of LDA in 1:1:1 heptane/THF/ethylbenzene (0.6 mL, 1.0 mmol). The reaction mixture was stirred under Ar at  $-78^{\circ}\text{C}$  for 45 min after which time a solution of tetrabenzylpyrophosphate (0.5 g, 1.0 mmol) in anhydrous THF (5.0 mL) was added. The solution was stirred at  $-78^{\circ}\text{C}$  under Ar for a further 4h and then concentrated *in vacuo* to give a yellow solid. This was dissolved in DCM (2.0 mL) and purified by column chromatography on silica using 4% MeOH in DCM as eluent to afford the product, **162**, (0.3 g, 75%) as a white foam.

$R_f$  (DCM:MeOH, 9:1) 0.62; Mp  $62.1\text{--}63.9^{\circ}\text{C}$ ;  $[\alpha]_D^{25} 29.9^{\circ}$  ( $c$  1.3,  $\text{CHCl}_3$ ) (Lit.  $[\alpha]_D^{25} 29.0^{\circ}$  ( $c$  1.0,  $\text{CHCl}_3$ )<sup>144</sup>); HRMS (FAB) 608.1894 ( $[\text{M}+\text{H}]^+$ ,  $\text{C}_{28}\text{H}_{35}\text{O}_{12}\text{NP}$  requires 608.1897); LRMS (FAB) 608 ( $\text{C}_{28}\text{H}_{35}\text{O}_{12}\text{NP}$ ), 300 ( $\text{C}_{14}\text{H}_{20}\text{O}_8\text{N}$ ), 91 ( $\text{C}_7\text{H}_7$ ), 43 ( $\text{C}_2\text{H}_3\text{O}$ );  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 250MHz) 7.57–7.45 (10H, m, ArH), 5.61 (1H, br d,  $J$  9.2 Hz, NH), 5.55 (1H, dd,  $J_{\text{H1-P}}$  6.0 Hz,  $J_{1,2}$  3.2 Hz, H1), 5.05 (1H, dd,  $J_{4,5}$  10.9 Hz,  $J_{4,3}$  9.9 Hz, H4), 5.00–4.94 (5H, complex m, 2xPOCH<sub>2</sub>, H2), 4.32–4.22 (1H, m, H3), 4.03 (1H, dd,  $J_{\text{H6b-H5}}$  3.8 Hz,  $J_{\text{H6b-H6a}}$  12.4 Hz, H6b), 3.93–3.85 (1H, m, H5), 3.80 (1H, dd,  $J_{\text{H6a-H6b}}$  12.4 Hz,  $J_{\text{H6a-H5}}$  2.2 Hz, H6a), 1.93, 1.92, 1.90 and 1.59 (4 x 3H, 4 x s, 4 x COCH<sub>3</sub>);  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 63MHz) 171.0, 170.4, 170.0 and 168.9 (CO), 135.2 and 135.1 (ArC), 128.8 (4 x ArCH), 128.7 (2 x ArCH), 128.0 (2 x ArCH), 127.9 (2 x ArCH), 96.1 and 69.9 (CH), 69.8 (2 x CH<sub>2</sub>), 69.5 and 67.1 (CH), 61.1 (CH<sub>2</sub>), 51.6 (CH), 22.6 and 20.5 (CH<sub>3</sub>), 20.4 (2 x CH<sub>3</sub>);  $\delta_{\text{P}}$  ( $\text{CDCl}_3$ , 101MHz) 0.0 (s, P=O);  $\nu_{\text{max}}$  (film)/ $\text{cm}^{-1}$  2982 (CH), 1747 (OC=O), 1684 (N-C=O), 1139 (P=O) and 1231 (P-O-Bn).



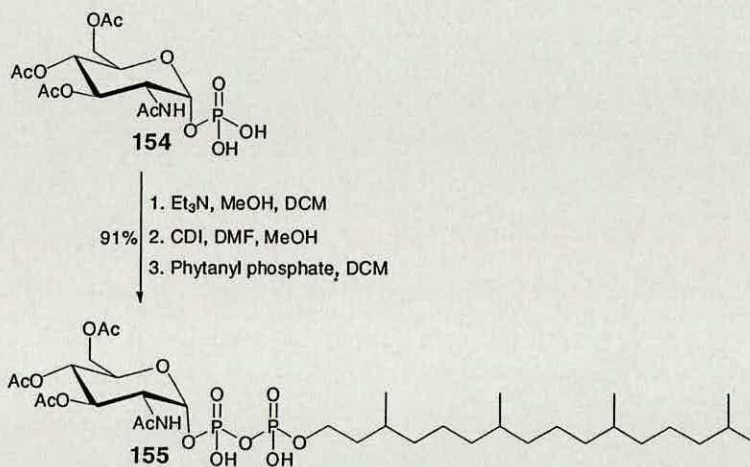
2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate, **154**.



A stirred solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate dibenzyl ester, **162**, (330.0 mg, 0.5 mmol) in 2:1 anhydrous DCM:methanol (15.0 mL) was treated with finely divided 10% palladium on charcoal (133.0 mg) as catalyst and placed under an atmosphere of  $N_2$ . The system was evacuated and flushed with hydrogen. This process was repeated twice and the reaction mixture was then allowed to stir under an atmosphere of hydrogen for 16h. The solution was filtered through a pad of celite, which was washed well with DCM (15.0 mL) and methanol (2 x 20.0 mL). The combined filtrate and washings were concentrated *in vacuo* to give a colourless residue. This was dried under high vacuum to afford the product, **154**, (0.2 g, 100%) as a white foam.

$R_f$  (DCM:MeOH:H<sub>2</sub>O:2M aq NH<sub>4</sub>HCO<sub>3</sub>, 6.5:3.5:0.4:0.4) 0.19; Mp dec 109.5-111.7 °C;  $[\alpha]_D^{25}$  71.2° (*c* 1.1, CHCl<sub>3</sub>); HRMS (FAB) 450.0783 ([M+Na]<sup>+</sup>, C<sub>14</sub>H<sub>22</sub>O<sub>12</sub>NPNa requires 450.0778); LRMS (FAB) 450 (C<sub>14</sub>H<sub>22</sub>O<sub>12</sub>NPNa), 330 (C<sub>14</sub>H<sub>20</sub>O<sub>8</sub>N), 289 (C<sub>12</sub>H<sub>19</sub>O<sub>7</sub>N), 43 (C<sub>2</sub>H<sub>3</sub>O); CHN found %C 39.12, %H 5.55, %N 2.95 (required %C 39.34, %H 5.19, %N 3.28);  $\delta_H$  (CDCl<sub>3</sub>, 250MHz) 7.52 (2H, br s, P(OH)<sub>2</sub>), 5.69 (1H, br s, NH), 5.25-5.22 (1H, m, CH), 5.16-5.07 (1H, m, CH), 4.54-4.23 (5H, br m, 5 x CH), 2.04-1.97 (12H, m, 4 x COCH<sub>3</sub>);  $\delta_C$  (CDCl<sub>3</sub>, 63MHz) 174.1, 171.1, 170.9 and 169.3 (CO), 94.1 69.8, 68.9 and 67.7 (CH), 61.4 (CH<sub>2</sub>), 52.3 (CH), 21.9 (2 x CH<sub>3</sub>), 20.6 and 20.4 (CH<sub>3</sub>);  $\delta_P$  (CDCl<sub>3</sub>, 101MHz) -7.9 (s, P=O);  $\nu_{max}$  (film)/cm<sup>-1</sup> 2986 (CH), 1750 (OC=O), 1603 (N-C=O), 1154 (P=O) and 1044 (P-OH).

***P*<sub>1</sub>-phytanyl-*P*<sub>2</sub>-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl pyrophosphate, 155.**



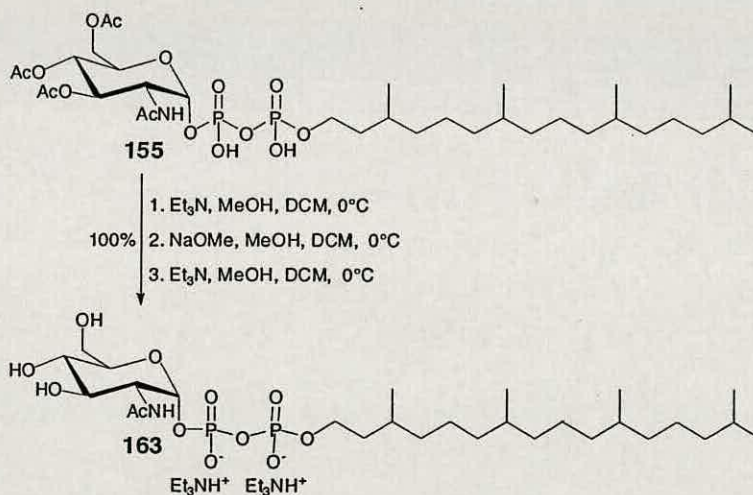
A stirred solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl phosphate, **154**, (230.9 mg, 0.5 mmol) in anhydrous methanol (6.0 mL) and DCM (0.2 mL) was treated with triethylamine (1.5 mL). The reaction mixture was stirred under N<sub>2</sub> at room temperature for 5 min. The solution was concentrated and azeotroped with anhydrous toluene (3 x 3.0 mL). The resulting pale yellow residue was dissolved in anhydrous DMF (3.0 mL) and treated with a solution of *N,N'*-carbonyldiimidazole (560.0 mg, 3.5 mmol) in anhydrous DMF (3.0 mL). The reaction mixture was stirred under N<sub>2</sub> at room temperature for 4.5h. Anhydrous methanol (0.5 mL) was added to the reaction, which was stirred under N<sub>2</sub> at room temperature for 30 min. The reaction mixture was then treated with a solution of phytanyl phosphate, **98**, (360.0 mg, 0.9 mmol) in anhydrous DCM (2.0 mL) and the reaction mixture was stirred under N<sub>2</sub> at room temperature for 4 days after which time the solution was concentrated *in vacuo* to yield a yellow oil. This was purified by column chromatography on silica using DCM:MeOH:H<sub>2</sub>O:2M aq NH<sub>4</sub>CO<sub>3</sub>, 9:1:0.1:0.1, as eluent to afford the product, **155**, (0.4 g, 91%) as a white foam.

R<sub>f</sub> (DCM:MeOH:H<sub>2</sub>O:2M aq NH<sub>4</sub>HCO<sub>3</sub>, 6.5:3.5:0.4:0.4) 0.67; [α]<sub>D</sub> 22.8° (*c* 0.8, CHCl<sub>3</sub>); HRMS (FAB) 810.3593 ([M+Na]<sup>+</sup>, C<sub>34</sub>H<sub>63</sub>O<sub>15</sub>NP<sub>2</sub>Na requires 810.3591);



LRMS (FAB) 810 ( $C_{34}H_{63}O_{15}NP_2Na$ ), 790 ( $C_{34}H_{66}O_{15}NP_2$ ), 379 ( $C_{20}H_{44}O_4P$ ), 330 ( $C_{14}H_{20}O_8N$ ), 43 ( $C_2H_3O$ );  $\delta_H$  ( $CDCl_3$ , 250MHz) 7.48 (2H, br s,  $P(OH)_2$ ), 7.19 (1H, dd,  $J_{3,2}$  9.8 Hz,  $J_{3,4}$  10.2 Hz, H3), 7.10 (1H, dd,  $J_{2,1}$  2.9 Hz,  $J_{2,3}$  9.8 Hz, H2), 6.24 (1H, br d,  $J$  10.5 Hz, NH), 6.14 (1H, dd,  $J_{H1-P}$  7.2 Hz,  $J_{1,2}$  2.9 Hz, H1), 5.09-5.03 (6H, br m,  $POCH_2$ , H4, H5, H6a, H6b), 4.00, 3.99, 3.93 and 3.91 (4 x 3H, 4 x s, 4 x  $COCH_3$ ), 3.48-3.41 (2H, m,  $CH_2$ ), 3.28-3.10 (18H, br m, 4 x CH, 7 x  $CH_2$ ), 3.08-2.97 (4H, m, 2 x  $CH_2$ ), 2.81-2.75 (15H, m, 5 x  $CH_3$ );  $\delta_C$  ( $CDCl_3$ , 63MHz) 170.9 (2 x CO), 170.5 and 169.5 (CO), 71.0 (CH), 68.2 (2 x CH), 67.9 (CH), 61.1 ( $CH_2$ ), 51.3 (CH), 45.6 (4 x  $CH_2$ ), 38.9 and 37.2 ( $CH_2$ ), 37.1 (2 x  $CH_2$ ), 32.4 (2 x CH), 29.3 and 27.6 (CH), 24.4, 24.1 and 22.2 ( $CH_2$ ), 22.1 (2 x  $CH_3$ ), 21.9 ( $CH_3$ ), 20.2 (2 x  $CH_3$ ), 20.1 (2 x  $CH_3$ ), 19.2 (2 x  $CH_3$ );  $\delta_P$  ( $CDCl_3$ , 101MHz) -7.9 (s,  $P=O$ ) and -11.0 (s,  $P=O$ );  $\nu_{max}$  (film)/ $cm^{-1}$  3019 (CH), 1742 (OC=O), 1058 (P-OH) and 1030 (P-O-P).

***P*<sub>1</sub>-phytanyl-*P*<sub>2</sub>-2-acetamido-2-deoxy- $\alpha$ -D-glucopyranosyl pyrophosphate, 163.**



A stirred solution of *P*<sub>1</sub>-phytanyl-*P*<sub>2</sub>-2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl pyrophosphate, **155**, (52.7 mg, 66.9  $\mu$ mol) in anhydrous DCM (2.0 mL) and anhydrous methanol (1.0 mL) was cooled to 0°C and treated with triethylamine (0.6 mL). The reaction was stirred under  $N_2$  for 5 min and then concentrated *in vacuo*. The resulting residue was azeotroped with anhydrous toluene (3 x 3.0 mL) and then re-dissolved in anhydrous DCM (2.0 mL) and methanol (1.0 mL). The reaction was cooled to 0°C, treated with 0.5 M sodium methoxide solution

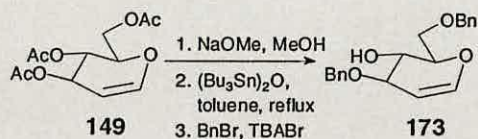
(100.0  $\mu$ L, 50.0  $\mu$ mol) and stirred under N<sub>2</sub> for 15 min. The reaction mixture was passed through a H<sup>+</sup> Dowex AG50W-X8 ion exchange resin, which was then washed with anhydrous methanol (7 x 1.0 mL). The combined filtrate and washings were concentrated *in vacuo* and azeotroped with anhydrous toluene (3 x 3.0 mL). The resulting residue was dissolved in anhydrous DCM (2.0 mL) and anhydrous methanol (1.0 mL) and cooled to 0°C, treated with triethylamine (0.6 mL) and stirred under N<sub>2</sub> for 5 min. The solution was concentrated *in vacuo* and azeotroped with anhydrous toluene (3 x 3.0 mL) to give a colourless residue. This was dried under high vacuum to afford the product, **163**, (44.1 mg, 100%) as a white foam.

R<sub>f</sub> (DCM:MeOH:H<sub>2</sub>O:2M aq NH<sub>4</sub>HCO<sub>3</sub>, 6.5:3.5:0.4:0.4) 0.31; [ $\alpha$ ]<sub>D</sub> 4.1° (c 0.3, CHCl<sub>3</sub>); *m/z* (FAB) 684.3257 ([M+Na]<sup>+</sup>, C<sub>28</sub>H<sub>57</sub>O<sub>12</sub>NP<sub>2</sub>Na requires 684.3254); LRMS (FAB) 684 (C<sub>28</sub>H<sub>57</sub>O<sub>12</sub>NP<sub>2</sub>Na), 662 (C<sub>28</sub>H<sub>58</sub>O<sub>12</sub>NP<sub>2</sub>), 379 (C<sub>20</sub>H<sub>44</sub>O<sub>4</sub>P);  $\delta$ <sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 5.56-5.49 (1H, m, CH), 4.22-4.11 (1H, m, CH), 4.00-3.79 (2H, m, 2 x CH), 3.18-2.97 (2H, m, 2 x CH), 2.01-1.92 (1H, m, CH), 1.32-0.99 (29H, br m, 2 x CH, 12 x CH<sub>2</sub>, COCH<sub>3</sub>), 0.81-0.75 (15H, m, 5 x CH<sub>3</sub>);  $\delta$ <sub>C</sub> (CDCl<sub>3</sub>, 63MHz) 171.4 (CO), 74.2, 72.2, 68.9 and 65.3 (CH), 62.0 (CH<sub>2</sub>), 51.9 (CH), 46.1, 39.8, 39.1 and 37.9 (CH<sub>2</sub>), 37.7 (2 x CH<sub>2</sub>), 33.2, 32.4 and 30.8 (CH), 30.6, 30.1 and 29.3 (CH<sub>2</sub>), 28.4 (CH), 24.8 and 24.1 (CH<sub>2</sub>), 23.4 (2 x CH<sub>3</sub>), 20.3 (CH<sub>3</sub>), 20.1, 19.4 and 18.9 (CH<sub>3</sub>);  $\delta$ <sub>P</sub> (CDCl<sub>3</sub>, 101MHz) -9.0 (s, P=O) and -11.9 (s, P=O).



#### 4.4. Synthesis of protected chitobiose.

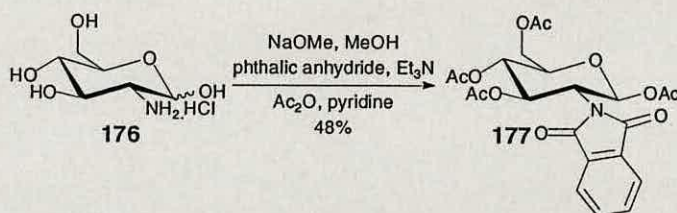
##### Di-*O*-benzyl-D-glucal, 173.



A stirred solution of tri-*O*-acetyl-D-glucal, **149**, (2.0 g, 7.4 mmol) in anhydrous MeOH (10.0 mL) was treated with sodium methoxide (0.1 g, 2.2 mmol). The reaction mixture was stirred under an atmosphere of N<sub>2</sub> for 2h at room temperature and then concentrated *in vacuo* to yield a pale yellow oil. This was purified by column chromatography on silica using 20% MeOH in DCM as the eluent to afford D-glucal (1.1 g, 100%) as a white foam. The D-glucal thus obtained was dissolved in anhydrous toluene (10.0 mL) and treated with *bis*-tributyl tin oxide (4.2 mL, 8.3 mmol). The reaction mixture was heated at reflux in a Dean-Stark apparatus for 16h. The pale yellow reaction mixture was allowed to cool to room temperature so that benzyl bromide (2.9 mL, 20.0 mmol) and tetrabutylammonium bromide (4.8 g, 15.0 mmol) could be added to the reaction mixture. The reaction was then heated at reflux in a Dean-Stark apparatus for a further 16h. The dark brown reaction mixture was cooled to room temperature, concentrated *in vacuo* and the resulting residue was dissolved in EtOAc (30.0 mL). The organic layer was washed with water (2 x 30.0 mL), a saturated solution of NaHCO<sub>3</sub> (40.0 mL) and brine (40.0 mL). The organic layer was dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a dark brown oil. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent but no product was isolated.



## 2-Phthalimido-2-deoxy-1,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranose, **177**.<sup>131</sup>



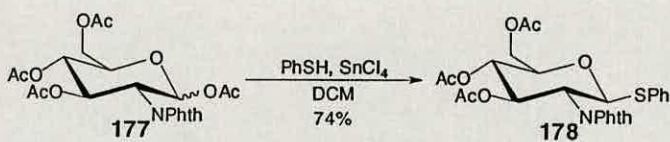
A stirred solution of glucosamine hydrochloride, **176**, (10.0 g, 46.4 mmol) in anhydrous methanol (80.0 mL) was treated with sodium methoxide (3.0 g, 46.4 mmol) and stirred under N<sub>2</sub> for 30 min at room temperature. The reaction mixture was filtered and the filtrate treated with phthalic anhydride (3.5 g, 23.2 mmol). The reaction was stirred under N<sub>2</sub> at room temperature for 20 min and further quantities of phthalic anhydride (3.5 g, 23.2 mmol) and triethylamine (7.6 mL, 55.6 mmol) were added. The creamy white solution was stirred under N<sub>2</sub> at room temperature for 10 min and then at 50°C for 30 min. The resulting mixture was cooled in an ice bath for 1h and then the white precipitate was removed by vacuum filtration and dried under vacuum for 4h. The dry white solid was dissolved in acetic anhydride (45.0 mL), cooled in an ice bath and slowly treated with pyridine (23.0 mL). The resulting yellow solution was stirred under N<sub>2</sub> at room temperature for 16h. The reaction mixture was diluted with DCM (200.0 mL) and poured onto a mixture of ice and water (200.0 mL). The organic layer was separated, washed with 5% aqueous HCl (150.0 mL), a saturated solution of NaHCO<sub>3</sub> (150.0 mL) and brine (150.0 mL). The organic layer was dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a yellow syrup. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent to afford the product, **177**, (10.5 g, 48%) as a white foam.

R<sub>f</sub> (petroleum ether:ethyl acetate, 6:4) 0.21; Mp 48.0-50.0 °C; [ $\alpha$ ]<sub>D</sub> 66.3° (*c* 1.2, CHCl<sub>3</sub>) (Lit [ $\alpha$ ]<sub>D</sub> 68.0 ° (*c* 0.5, CHCl<sub>3</sub>)<sup>145</sup>); HRMS (FAB) 478.1349 ([M+H]<sup>+</sup>, C<sub>22</sub>H<sub>24</sub>O<sub>11</sub>N requires 478.1349);  $\delta$ <sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 7.91-7.76 (4H, m, ArH, NPhth), 6.54 (1H, d, *J*<sub>1,2</sub> 8.9 Hz, H1), 5.91 (1H, dd, *J*<sub>3,2</sub> 10.6 Hz, *J*<sub>3,4</sub> 9.2 Hz, H3),



5.24 (1H, dd,  $J_{4,5}$  10.2 Hz,  $J_{4,3}$  9.2 Hz, H4), 4.49 (1H, dd,  $J_{2,3}$  10.6 Hz,  $J_{2,1}$  8.9 Hz, H2), 4.40 (1H, dd,  $J_{H6b-H6a}$  12.5 Hz,  $J_{H6b-H5}$  4.4 Hz, H6b), 4.18 (1H, dd,  $J_{H6a-H6b}$  12.5 Hz,  $J_{H6a-H5}$  2.1 Hz, H6a), 4.04-3.97 (1H, m, H5), 2.14, 2.07, 2.03 and 1.89 (4 x 3H, 4 x s, 4 x COCH<sub>3</sub>);  $\delta_c$  (CDCl<sub>3</sub>, 63MHz) 171.2 and 170.5 (CO), 169.9 (2 x CO), 169.1 and 167.9 (CO), 134.9 (2 x ArCH), 131.7 (2 x ArC), 124.3 (2 x ArCH), 90.2, 73.1, 70.9 and 68.7 (CH), 61.9 (CH<sub>2</sub>), 53.9 (CH), 21.2 (2 x CH<sub>3</sub>), 21.1 and 20.9 (CH<sub>3</sub>);  $\nu_{max}$  (film)/cm<sup>-1</sup> 1755, 1722 (OC=O), 1550 (Ph).

**2-Phthalimido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **178**.<sup>146</sup>**



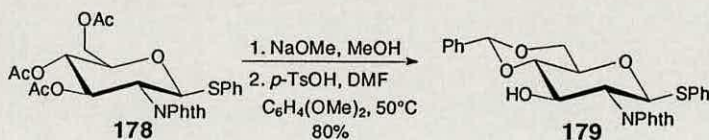
A stirred solution of 2-phthalimido-2-deoxy-1,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranose, **177**, (21.4 g, 44.9 mmol) in anhydrous DCM (200.0 mL) was treated with benzenethiol (10.5 mL, 100.0 mmol) and tin tetrachloride (10.0 mL, 15.6 mmol). The dark brown reaction mixture was stirred under N<sub>2</sub> at room temperature for 16h. The reaction was then diluted with DCM (100.0 mL) and washed with a saturated solution of K<sub>2</sub>CO<sub>3</sub> (2 x 150.0 mL). The organic extract was washed with brine (100.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a yellow syrup. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent to afford the product, **178**, (17.4 g, 74%) as a white foam.

$R_f$  (petroleum ether:ethyl acetate, 6:4) 0.24; Mp 141.0-142.5 °C (Lit 145.0-146.0 °C<sup>146</sup>);  $[\alpha]_D^{25}$  53.0° ( $c$  1.2, CHCl<sub>3</sub>) (Lit  $[\alpha]_D^{25}$  53.0° ( $c$  1.4, CHCl<sub>3</sub>)<sup>146</sup>); HRMS (FAB) 528.1314 ([M+H]<sup>+</sup>, C<sub>26</sub>H<sub>26</sub>O<sub>9</sub>NS requires 528.1318); CHN found %C 58.93, %H 4.78, %N 2.73 (required %C 59.20, %H 4.74, %N 2.66);  $\delta_H$  (CDCl<sub>3</sub>, 250MHz) 7.91-7.78 (4H, m, ArH, NPhth), 7.46-7.29 (5H, m, ArH, SPh), 5.83 (1H, dd,  $J_{3,2}$  10.2 Hz,



$J_{3,4}$  9.2 Hz, H3), 5.75 (1H, d,  $J_{1,2}$  10.6 Hz, H1), 5.17 (1H, dd,  $J_{4,5}$  10.2 Hz,  $J_{4,3}$  9.2 Hz, H4), 4.38 (1H, dd,  $J_{2,3}$  10.2 Hz,  $J_{2,1}$  10.6 Hz, H2), 4.32 (1H, dd,  $J_{H6b-H6a}$  12.3 Hz,  $J_{H6b-H5}$  5.1 Hz, H6b), 4.24 (1H, dd,  $J_{H6a-H6b}$  12.3 Hz,  $J_{H6a-H5}$  2.4 Hz, H6a), 3.96-3.91 (1H, m, H5), 2.13, 2.05 and 1.87 (3 x 3H, 3 x s, 3 x COCH<sub>3</sub>);  $\delta_c$  (CDCl<sub>3</sub>, 63MHz) 171.1 (qC), 170.5 (2 x qC), 169.9, 168.2, 167.4 and 162.9 (qC), 134.9 and 134.8 (ArCH), 133.7 (2 x ArCH), 131.4 (ArC), 129.3 (2 x ArCH), 128.8 (ArCH), 124.1 (2 x ArCH), 83.5, 76.3, 72.0 and 69.1 (CH), 62.6 (CH<sub>2</sub>), 53.9 (CH), 21.2, 21.0 and 20.8 (CH<sub>3</sub>);  $\nu_{max}$  (film)/cm<sup>-1</sup> 2986 (CH), 1750 (OC=O), 1610 (NC=O), 1550 (Ph).

**2-Phthalimido-2-deoxy-4,6-*O*-benzylidene-1-thiophenyl- $\beta$ -D-glucopyranoside, **179**.<sup>146</sup>**

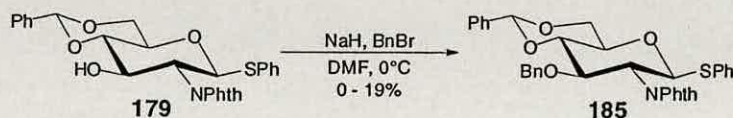


A stirred solution of 2-phthalimido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **178**, (3.8 g, 7.2 mmol) in anhydrous methanol (50.0 mL) was treated with sodium methoxide (20.0 mg, 0.7 mmol). The reaction was stirred under N<sub>2</sub> at room temperature for 1h and then neutralised with glacial acetic acid (0.1 mL). The solution was concentrated *in vacuo* to yield a pale yellow foam which was then azeotroped with anhydrous toluene (3 x 30.0 mL). The resulting residue was dried under vacuum for 1h, then dissolved in anhydrous DMF (50.0 mL) and treated with benzaldehyde dimethyl acetal (2.5 mL, 16.5 mmol) and *p*-toluenesulfonic acid (0.3 g, 1.6 mmol). The reaction was stirred under N<sub>2</sub> for 1h at 50°C and then cooled to room temperature. The reaction was diluted with toluene (50.0 mL) and washed with a saturated solution of NaHCO<sub>3</sub> (2 x 50.0 mL). The organic layer was washed with brine (50.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a yellow syrup. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent to afford the product, **179**, (5.4 g, 80%) as a white foam.



$R_f$  (petroleum ether:ethyl acetate, 6:4) 0.29; Mp 61.3-63.6 °C;  $[\alpha]_D^{25}$  11.7° ( $c$  1.0,  $\text{CHCl}_3$ ) (Lit 34.2° ( $c$  1.3,  $\text{CHCl}_3$ )<sup>146</sup>); HRMS (FAB) 490.1323 ( $[\text{M}+\text{H}]^+$ ,  $\text{C}_{27}\text{H}_{24}\text{O}_6\text{NS}$  requires 490.1324);  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 250MHz) 7.94-7.90 (4H, m, ArH, NPhth), 7.44-7.36 (5H, m, ArH), 7.31-7.24 (5H, m, ArH), 5.74 (1H, d,  $J_{1,2}$  10.5 Hz, H1), 5.60 (1H, s,  $\text{CH}(\text{O})_2$ ), 4.68 (1H, m, H3), 4.44 (1H, dd,  $J_{\text{H6b-H6a}}$  10.3 Hz,  $J_{\text{H6b-H5}}$  4.7 Hz, H6b), 4.35 (1H, dd,  $J_{2,3}$  9.5 Hz,  $J_{2,1}$  10.5 Hz, H2), 4.21-4.16 (1H, m, H6a), 3.75 (1H, m, H5), 3.65 (1H, dd,  $J_{4,5}$  9.4 Hz,  $J_{4,3}$  4.1 Hz, H4), 2.99 (1H, br s, OH);  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 63MHz) 168.7 and 168.0 (qC), 137.3 (2 x qC), 136.8 (2 x qC), 134.9, 134.7, 134.6, 133.1, 132.6, 132.2, 132.0, 130.2, 129.8, 129.4, 129.3, 128.8, 128.6 and 126.8 (ArCH), 102.4 ( $\text{CH}(\text{O})_2$ ), 84.7, 82.3, 70.8 and 70.1 (CH), 69.0 ( $\text{CH}_2$ ), 56.1 (CH);  $\nu_{\text{max}}$  (film)/ $\text{cm}^{-1}$  3412 (OH), 2967 (CH), 1610 ( $\text{NC}=\text{O}$ ), 1362 ( $\text{C}(\text{O})_2$ ).

**2-Phthalimido-2-deoxy-3-*O*-benzyl-4,6-*O*-benzylidene-1-thiophenyl- $\beta$ -D-glucopyranoside, **185**.<sup>147</sup>**

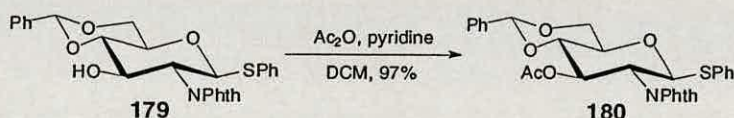


A stirred solution of 2-phthalimido-2-deoxy-4,6-*O*-benzylidene-1-thiophenyl- $\beta$ -D-glucopyranoside, **179**, (0.3 g, 0.5 mmol) in anhydrous DMF (5.0 mL) was cooled to 0°C in an ice bath and treated with sodium hydride (20.0 mg, 0.8 mmol). The reaction mixture was stirred under an atmosphere of  $\text{N}_2$  at 0°C while benzyl bromide (90.0  $\mu\text{L}$ , 0.8 mmol) was added in a dropwise manner over a 2h period. When the addition was complete the pale yellow reaction mixture was stirred under  $\text{N}_2$  at room temperature for 16h. The solution was diluted with methanol (2.0 mL) and then concentrated *in vacuo*. The resulting residue was dissolved in DCM (10.0 mL) and washed with a saturated solution of  $\text{NaHCO}_3$  (2 x 15.0 mL) and brine (15.0 mL). The organic layer was dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a yellow syrup. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 9:1, as the eluent to afford the product, **185**, (67.6 mg, 19%) as a white foam.



$R_f$  (petroleum ether:ethyl acetate, 6:4) 0.53; HRMS (FAB) 580.1803 ( $[M+H]^+$ ,  $C_{34}H_{30}O_6NS$  requires 580.1794);  $\delta_H$  ( $CDCl_3$ , 250MHz) 7.86-7.61 (4H, m, ArH, NPhth), 7.52-7.38 (5H, m, ArH), 7.36-7.21 (5H, m, ArH), 6.98-6.85 (5H, m, ArH), 5.62 (1H, d,  $J_{1,2}$  10.4 Hz, H1), 5.61 (1H, s,  $CH(O)_2$ ), 4.74 (1H, dd,  $J_{H6b-H6a}$  12.2 Hz,  $J_{H6b-H5}$  3.7 Hz, H6b), 4.50 (2H, s,  $PhCH_2O$ ), 4.45 (1H, dd,  $J_{3,2}$  10.2 Hz,  $J_{3,4}$  9.5 Hz, H3), 4.39 (1H, dd,  $J_{4,3}$  9.5 Hz,  $J_{4,5}$  10.3 Hz, H4), 4.27 (1H, dd,  $J_{2,1}$  10.4 Hz,  $J_{2,3}$  10.2 Hz, H2), 3.75 (1H, dd,  $J_{H6a-H6b}$  12.2 Hz,  $J_{H6a-H5}$  2.5 Hz, H6a), 3.73-3.68 (1H, m, H5);  $\delta_C$  ( $CDCl_3$ , 63MHz) 167.6 and 167.1 (CO), 137.5 and 137.1 (ArC), 133.9 and 133.7 (ArCH), 132.6 (3 x ArC), 128.9 and 128.8 (ArCH), 128.4 and 128.2 (2 x ArCH), 128.0 (2 x ArCH), 127.9 (2 x ArCH), 127.8, 127.5, 127.3, 126.9, 126.2, 125.9, 123.4 and 123.3 (ArCH), 101.2 ( $CH(O)_2$ ), 83.9, 82.7 and 75.3 (CH), 74.1 ( $CH_2$ ), 70.2 and 68.5 (CH), 67.9 ( $CH_2$ ).

**2-Phthalimido-2-deoxy-3-*O*-acetyl-4,6-*O*-benzylidene-1-thiophenyl- $\beta$ -D-glucopyranoside, **180**.**

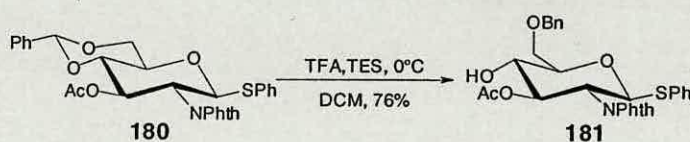


A stirred solution of 2-phthalimido-2-deoxy-4,6-*O*-benzylidene-1-thiophenyl- $\beta$ -D-glucopyranoside, **179**, (4.7 g, 9.6 mmol) in anhydrous DCM (20.0 mL) was treated with acetic anhydride (20.0 mL) and pyridine (10.0 mL). The pale yellow reaction mixture was stirred under  $N_2$  at room temperature for 16h. The solution was diluted with DCM (40.0 mL) and washed with water (50.0 mL), 5% aqueous HCl (50.0 mL), a saturated solution of  $NaHCO_3$  (50.0 mL) and brine (50.0 mL). The organic layer was dried over anhydrous  $MgSO_4$ , filtered and concentrated *in vacuo* to yield a yellow syrup. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent to afford the product, **180**, (4.9 g, 97%) as a white foam.



$R_f$  (petroleum ether:ethyl acetate, 6:4) 0.42; Mp 90.1-92.3 °C;  $[\alpha]_D^{25}$  22.9° ( $c$  1.0,  $\text{CHCl}_3$ ); HRMS (FAB) 532.1430 ( $[\text{M}+\text{H}]^+$ ,  $\text{C}_{29}\text{H}_{26}\text{O}_7\text{NS}$  requires 532.1430); LRMS (FAB) 554 ( $\text{C}_{29}\text{H}_{25}\text{O}_7\text{NSNa}$ ), 532 ( $\text{C}_{29}\text{H}_{26}\text{O}_7\text{NS}$ ), 422 ( $\text{C}_{23}\text{H}_{20}\text{O}_7\text{N}$ ); CHN found %C 65.26, %H 5.03, %N 2.36 (required %C 65.52, %H 4.71, %N 2.64);  $\delta_H$  ( $\text{CDCl}_3$ , 250MHz) 7.87-7.72 (4H, m, ArH, NPhth), 7.44-7.36 (5H, m, ArH), 7.35-7.24 (5H, m, ArH), 5.89 (1H, dd,  $J_{3,2}$  9.7 Hz,  $J_{3,4}$  9.2 Hz, H3), 5.82 (1H, d,  $J_{1,2}$  10.6 Hz, H1), 5.53 (1H, s,  $\text{CH}(\text{O})_2$ ), 4.43 (1H, dd,  $J_{4,5}$  9.7 Hz,  $J_{4,3}$  9.2 Hz, H4), 4.35 (1H, dd,  $J_{2,3}$  9.7 Hz,  $J_{2,1}$  10.6 Hz, H2), 3.84-3.81 (1H, m, H5), 3.79 (1H, dd,  $J_{\text{H6b-H6a}}$  9.7 Hz,  $J_{\text{H6b-H5}}$  4.3 Hz, H6b), 3.77-3.73 (1H, m, H6a), 1.86 (3H, s,  $\text{COCH}_3$ );  $\delta_C$  ( $\text{CDCl}_3$ , 63MHz) 170.1, 167.7, 167.1, 166.3 and 136.7 (qC), 134.4 and 134.1 (ArCH), 132.9 (2 x ArCH), 131.5 and 130.9 (qC), 129.1 (ArCH), 128.9 (2 x ArCH), 128.3 (ArCH), 128.1 (2 x ArCH), 126.1 (2 x ArCH), 123.6 and 123.5 (ArCH), 101.5 ( $\text{CH}(\text{O})_2$ ), 83.7, 78.8, 70.4 and 70.3 (CH), 68.4 ( $\text{CH}_2$ ), 54.1 (CH), 20.4 ( $\text{CH}_3$ );  $\nu_{\text{max}}$  (film)/ $\text{cm}^{-1}$  2986 (CH), 1719 ( $\text{OC}=\text{O}$ ), 1384 ( $\text{C}(\text{O})_2$ ).

## 2-Phthalimido-2-deoxy-3-*O*-acetyl-6-benzyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **181**.

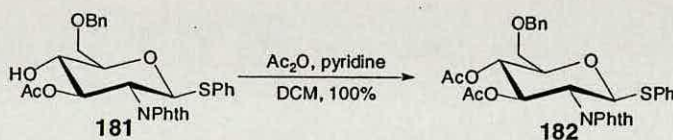


A stirred solution of 2-phthalimido-2-deoxy-3-*O*-acetyl-4,6-*O*-benzylidene-1-thiophenyl- $\beta$ -D-glucopyranoside, **180**, (5.7 g, 10.6 mmol) in anhydrous DCM (40.0 mL) was treated with triethylsilane (8.4 mL, 52.9 mmol) and the reaction mixture was cooled to 0°C under  $\text{N}_2$ . Trifluoroacetic acid (4.1 mL, 52.9 mmol) was added, the solution was gradually allowed to warm to room temperature and then stirred under  $\text{N}_2$  for 4h. The reaction was diluted with DCM (30.0 mL) and washed with a saturated solution of  $\text{NaHCO}_3$  (2 x 40.0 mL). The organic layer was washed with brine (40.0 mL), dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a yellow syrup. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent to afford the product, **181**, (4.3 g, 76%) as a white foam.



$R_f$  (petroleum ether:ethyl acetate, 6:4) 0.24; Mp 44.5-46.8 °C;  $[\alpha]_D^{25}$  31.1° ( $c$  1.1,  $\text{CHCl}_3$ ); HRMS (FAB) 534.1589 ( $[\text{M}+\text{H}]^+$ ,  $\text{C}_{29}\text{H}_{28}\text{O}_7\text{NS}$  requires 534.1587); LRMS (FAB) 556 ( $\text{C}_{29}\text{H}_{27}\text{O}_7\text{NSNa}$ ), 534 ( $\text{C}_{29}\text{H}_{28}\text{O}_7\text{NS}$ ), 424 ( $\text{C}_{23}\text{H}_{22}\text{O}_7\text{N}$ );  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 250MHz) 7.95-7.77 (4H, m, ArH, NPhth), 7.50-7.41 (5H, m, ArH), 7.34-7.28 (5H, m, ArH), 5.86 (1H, d,  $J_{1,2}$  10.5 Hz, H1), 5.80-5.73 (1H, m, H3), 4.68 (2H, d,  $J_{\text{A,B}}$  11.8 Hz,  $\text{CH}_2$ ), 4.38 (1H, dd,  $J_{2,3}$  9.5 Hz,  $J_{2,1}$  10.5 Hz, H2), 3.95-3.87 (4H, complex m, H4, H5, H6a, H6b), 1.98 (3H, s,  $\text{COCH}_3$ );  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ , 63MHz) 171.6 (2 x qC), 168.3, 167.7 and 138.2 (qC), 134.9, 134.7 and 133.4 (ArCH), 133.2 (2 x ArCH), 132.1 (2 x qC), 129.3 (2 x ArCH), 128.9 (2 x ArCH), 128.5 (2 x ArCH), 128.3, 128.2 and 124.2 (ArCH), 83.6, 78.7 and 74.7 (CH), 74.2 ( $\text{CH}_2$ ), 71.5 (CH), 70.7 ( $\text{CH}_2$ ), 53.9 (CH), 21.1( $\text{CH}_3$ );  $\nu_{\text{max}}$  (film)/ $\text{cm}^{-1}$  3600 (OH), 2986 (CH), 1719 ( $\text{OC}=\text{O}$ ).

**2-Phthalimido-2-deoxy-3,4-*O*-acetyl-6-benzyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **182**.**

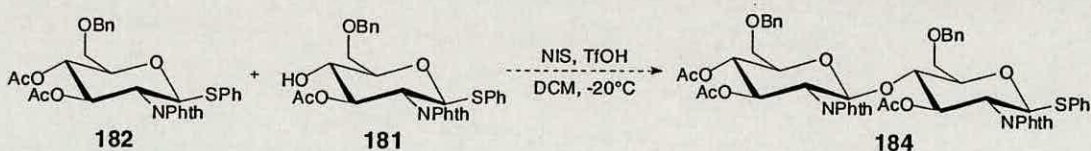


A stirred solution of 2-phthalimido-2-deoxy-3-*O*-acetyl-6-benzyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **181**, (0.6 g, 1.1 mmol) in anhydrous DCM (5.0 mL) was treated with acetic anhydride (6.0 mL) and pyridine (3.0 mL). The pale yellow reaction mixture was stirred under  $\text{N}_2$  at room temperature for 16h. The solution was diluted with DCM (20.0 mL) and washed with water (20.0 mL), 5% aqueous HCl (20.0 mL), a saturated solution of  $\text{NaHCO}_3$  (20.0 mL) and brine (20.0 mL). The organic layer was dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated *in vacuo* to yield a yellow syrup. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent to afford the product, **182**, (0.6 g, 100%) as a white foam.



R<sub>f</sub> (petroleum ether:ethyl acetate, 6:4) 0.35; Mp 111.0-113.4 °C; [ $\alpha$ ]<sub>D</sub> 79.6° (*c* 0.3, CHCl<sub>3</sub>); HRMS (FAB) 576.1698 ([M+H]<sup>+</sup>, C<sub>31</sub>H<sub>30</sub>O<sub>8</sub>NS requires 576.1692); LRMS (FAB) 598 (C<sub>31</sub>H<sub>29</sub>O<sub>8</sub>NSNa), 576 (C<sub>31</sub>H<sub>30</sub>O<sub>8</sub>NS), 466 (C<sub>25</sub>H<sub>24</sub>O<sub>8</sub>N);  $\delta$ <sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 7.89-7.74 (4H, m, ArH, NPhth), 7.44-7.21 (10H, m, ArH), 5.83 (1H, dd, *J*<sub>3,2</sub> 9.8 Hz, *J*<sub>3,4</sub> 9.2 Hz, H3), 5.74 (1H, d, *J*<sub>1,2</sub> 10.6 Hz, H1), 5.16 (1H, dd, *J*<sub>4,3</sub> 9.2 Hz, *J*<sub>4,5</sub> 9.2 Hz, H4), 4.56 (2H, q, *J*<sub>A,B</sub> 11.8 Hz, CH<sub>2</sub>), 4.37 (1H, dd, *J*<sub>2,3</sub> 9.8 Hz, *J*<sub>2,1</sub> 10.6 Hz, H2), 3.95-3.86 (1H, m, H5), 3.65 (2H, d, *J* 4.1 Hz, H6a, H6b), 1.95 and 1.84 (2 x 3H, 2 x s, 2 x COCH<sub>3</sub>);  $\delta$ <sub>C</sub> (CDCl<sub>3</sub>, 63MHz) 163.4 (2 x qC), 162.8 (2 x qC), 161.0, 160.2 and 131.0 (qC), 127.6 (ArCH), 126.5 (2 x ArCH), 126.0 (ArCH), 124.6 (qC), 122.1 (2 x ArCH), 121.6 (2 x ArCH), 121.4 (ArCH), 121.0 (2 x ArCH), 120.9 (ArCH), 116.9 (2 x ArCH), 76.3 and 70.7 (CH), 66.7 (CH<sub>2</sub>), 64.9 and 62.7 (CH), 62.2 (CH<sub>2</sub>), 46.8 (CH), 13.9 and 13.7 (CH<sub>3</sub>);  $\nu$ <sub>max</sub> (film)/cm<sup>-1</sup> 2986 (CH), 1719 (OC=O).

***O*-(2-Phthalimido-2-deoxy-3,4-*O*-acetyl-6-benzyl- $\beta$ -D-glucopyranosyl)-(1,4)-2-phthalimido-2-deoxy-3-*O*-acetyl-6-benzyl-1-thiophenyl- $\beta$ -D-glucopyranoside, 184.**

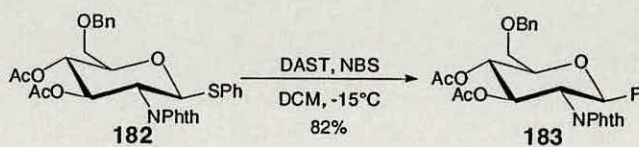


A stirred solution of 2-phthalimido-2-deoxy-3-*O*-acetyl-6-benzyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **181**, (66.0 mg, 0.1 mmol) in anhydrous DCM (5.0 mL) was cooled to -20°C and treated with a solution of 2-phthalimido-2-deoxy-3,4-*O*-acetyl-6-benzyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **182**, (124.1 mg, 0.2 mmol) in anhydrous DCM (5.0 mL), NIS (0.1 g, 0.6 mmol) and *p*-toluenesulfonic acid (11.0  $\mu$ L, 0.1 mmol). The reaction was stirred, while being protected from light, at -20°C under N<sub>2</sub> for 30 min. The solution was gradually allowed to warm to room temperature and stirred for 16h. The dark red reaction mixture was diluted with DCM (15.0 mL), washed with water (20.0 mL), a saturated solution of NaHCO<sub>3</sub> (20.0 mL) and brine (20.0 mL). The organic layer was dried over anhydrous MgSO<sub>4</sub>, filtered and



concentrated *in vacuo* to yield a brown syrup. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent but no product was isolated.

**2-Phthalimido-2-deoxy-3,4-*O*-acetyl-6-benzyl- $\beta$ -D-glucopyranosyl fluoride, 183.**



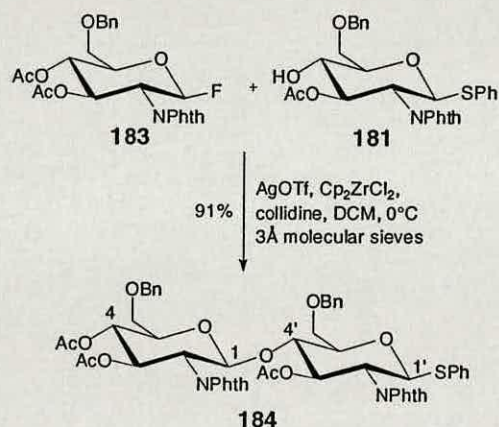
A stirred solution of 2-phthalimido-2-deoxy-3,4-*O*-acetyl-6-benzyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **182**, (2.1 g, 3.6 mmol) in anhydrous DCM (20.0 mL) was cooled to -15°C and treated with DAST (960.0  $\mu$ L, 7.3 mmol). The reaction was stirred under N<sub>2</sub> for 2 min before the addition of NBS (0.1 g, 5.5 mmol). The solution was gradually allowed to warm to room temperature and then stirred under N<sub>2</sub> for 16h. The reaction was diluted with DCM (10.0 mL) and washed with a saturated solution of NaHCO<sub>3</sub> (3 x 20.0 mL). The organic layer was washed with brine (20.0 mL), dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a yellow syrup. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 8:2, as the eluent to afford the product, **183**, (1.4 g, 82%) as a white foam.

R<sub>f</sub> (petroleum ether:ethyl acetate, 6:4) 0.11; Mp 52.2-54.4 °C; [ $\alpha$ ]<sub>D</sub> 37.3° (*c* 1.0, CHCl<sub>3</sub>); HRMS (FAB) 485.1574 ([M]<sup>+</sup>, C<sub>25</sub>H<sub>24</sub>O<sub>8</sub>NF requires 485.1476); LRMS (FAB) 508 (C<sub>25</sub>H<sub>24</sub>O<sub>8</sub>NFNa), 485 (C<sub>25</sub>H<sub>24</sub>O<sub>8</sub>NF), 466 (C<sub>25</sub>H<sub>24</sub>O<sub>8</sub>N);  $\delta$ <sub>H</sub> (CDCl<sub>3</sub>, 250MHz) 7.93-7.78 (4H, m, ArH, NPhth), 7.41-7.34 (5H, m, ArH), 6.13 (1H, br dd, *J*<sub>1,2</sub> 7.8 Hz, *J*<sub>F-H1</sub> 52.5 Hz, H1), 5.93 (1H, dd, *J*<sub>3,2</sub> 9.8 Hz, *J*<sub>3,4</sub> 9.1 Hz, H3), 5.30 (1H, dd, *J*<sub>4,3</sub> 9.1 Hz, *J*<sub>4,5</sub> 9.1 Hz, H4), 4.63 (2H, q, *J*<sub>A,B</sub> 12.0 Hz, CH<sub>2</sub>), 4.54-4.44 (1H, m, H2), 4.05-3.97 (1H, m, H5), 3.70-3.68 (2H, m, H6a, H6b), 1.97 and 1.91 (2 x 3H, 2 x s, 2 x COCH<sub>3</sub>);  $\delta$ <sub>C</sub> (CDCl<sub>3</sub>, 63MHz) 170.5, 170.4, 169.8, 169.1, 167.9 and 137.9 (qC), 134.9 and 132.8 (ArCH), 131.7 (qC), 130.9, 129.8, 129.1, 128.9, 128.4, 128.3 and 124.2 (ArCH), 90.2 (CH), 74.1 (CH<sub>2</sub>), 73.7 (CH), 70.5 (d, CH), 69.5 (CH), 68.6



(CH<sub>2</sub>), 61.9 (d, CH), 21.0 and 20.8 (CH<sub>3</sub>);  $\nu_{\max}$  (film)/cm<sup>-1</sup> 3004, 2967, 2924 (CH), 1714 (OC=O), 1362 (CF).

***O*-(2-Phthalimido-2-deoxy-3,4-*O*-acetyl-6-benzyl- $\beta$ -D-glucopyranosyl)-(1,4)-2-phthalimido-2-deoxy-3-*O*-acetyl-6-benzyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **184**.**



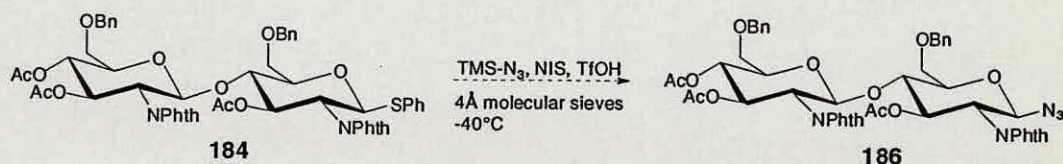
A stirred solution of silver trifluoromethanesulfonate (145.0 mg, 0.6 mmol), zirconocene dichloride (165.0 mg, 0.6 mmol), collidine (15.0  $\mu$ L, 0.1 mmol) and 3Å molecular sieves in anhydrous DCM (3.0 mL) was cooled to 0°C and stirred, while being protected from light, under N<sub>2</sub> for 10 min. A solution of 2-phthalimido-2-deoxy-3,4-*O*-acetyl-6-benzyl- $\beta$ -D-glucopyranosyl fluoride, **183**, (96.0 mg, 0.2 mmol) and 2-phthalimido-2-deoxy-3-*O*-acetyl-6-benzyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **181**, (61.0 mg, 0.1 mmol) in anhydrous DCM (3.0 mL) was added and the reaction mixture was stirred, while being protected from light, at 0°C under N<sub>2</sub>. The solution was gradually allowed to warm to room temperature and stirred for 16h. The grey reaction mixture was filtered and the filtrate concentrated *in vacuo* to yield a yellow oil. This was purified by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent to afford the product, **184**, (0.1 g, 91%) as a white foam.

R<sub>f</sub> (petroleum ether:ethyl acetate, 6:4) 0.19; Mp 79.0-80.9 °C; [ $\alpha$ ]<sub>D</sub> 38.4° (c 1.0, CHCl<sub>3</sub>); HRMS (FAB) 999.3020 ([M+H]<sup>+</sup>, C<sub>54</sub>H<sub>51</sub>O<sub>15</sub>N<sub>2</sub>S requires 999.3010);



LRMS (FAB) 1022 ( $C_{54}H_{51}O_{15}N_2SNa$ ), 999 ( $C_{54}H_{51}O_{15}N_2S$ ), 889 ( $C_{48}H_{45}O_{15}N_2$ ), 534 ( $C_{29}H_{28}O_7NS$ ), 466 ( $C_{25}H_{24}O_8N$ ), 424 ( $C_{23}H_{22}O_7N$ ); CHN found %C 61.78, %H 4.89, %N 2.75 (required %C 64.91, %H 5.01, %N 2.80);  $\delta_H$  ( $CDCl_3$ , 250MHz) 7.78-7.64 (8H, m, ArH, NPhth), 7.32-7.09 (15H, m, ArH), 5.77 (1H, dd,  $J_{3,2}$  9.7 Hz,  $J_{3,4}$  9.2 Hz, H3), 5.65 (1H, dd,  $J_{2',3'}$  10.8 Hz,  $J_{3',4'}$  10.4 Hz, H3'), 5.51 (1H, d,  $J_{1',2'}$  10.5 Hz, H1'), 5.39 (1H, d,  $J_{1,2}$  8.4 Hz, H1), 5.11 (1H, dd,  $J_{4,5}$  9.4 Hz,  $J_{4,3}$  9.2 Hz, H4), 4.54 (1H, dd,  $J_{4',5'}$  11.9 Hz,  $J_{4',3'}$  10.4 Hz, H4'), 4.43 (1H, dd,  $J_{H6a-H6b}$  12.0 Hz,  $J_{H6a-H5}$  2.9 Hz, H6a), 4.35 (2H, d,  $J$  8.4 Hz,  $CH_2$ ), 4.27 (2H, d,  $J$  5.9 Hz,  $CH_2$ ), 4.19 (1H, dd,  $J_{H6a'-H6b'}$  12.0 Hz,  $J_{H6a'-H5'}$  3.4 Hz, H6a'), 4.05 (1H, dd,  $J_{2,1}$  8.4 Hz,  $J_{2,3}$  9.7 Hz, H2), 3.81-3.75 (1H, m, H2'), 3.74-3.70 (1H, m, H5), 3.49 (2H, dd,  $J_{H6a-H6b}$  12.0 Hz,  $J_{H6b-H5}$  3.8 Hz, H6b', H6b), 3.42-3.36 (1H, m, H5'), 1.84-1.74 (9H, m, 3 x  $COCH_3$ );  $\delta_C$  ( $CDCl_3$ , 63MHz) 170.9 (qC), 170.0 (2 x qC), 169.5 (qC), 169.3 (2 x qC), 167.6, 167.1, 138.1 and 137.4 (qC), 134.2 (2 x ArCH), 132.9 and 132.6 (ArCH), 131.5 (2 x qC), 131.2 (2 x qC), 128.8 (2 x ArCH), 128.6 (2 x ArCH), 128.3 (2 x ArCH), 128.2 (2 x ArCH), 128.1 (ArCH), 127.9 (2xArCH), 127.8 (2xArCH), 127.7 (ArCH), 127.6 (2xArCH), 127.3 (ArCH), 123.5 (2 x ArCH), 96.9, 92.4, 82.8, 78.4, 74.1 and 73.5 (CH), 72.6 and 70.9 ( $CH_2$ ), 70.7 and 69.4 (CH), 68.2 and 67.6 ( $CH_2$ ), 54.9 and 53.4 (CH), 20.5, 20.4 and 20.3 ( $CH_3$ );  $\nu_{max}$  (film)/ $cm^{-1}$  3004, 2967, 2924 (CH), 1714 (OC=O), 1092 (C-O-C).

***O*-(2-Phthalimido-2-deoxy-3,4-*O*-acetyl-6-benzyl- $\beta$ -D-glucopyranosyl)-(1,4)-2-phthalimido-2-deoxy-3-*O*-acetyl-6-benzyl-1-azido- $\beta$ -D-glucopyranoside, 186.**



A stirred solution of *O*-(2-phthalimido-2-deoxy-3,4-*O*-acetyl-6-benzyl- $\beta$ -D-glucopyranosyl)-(1,4)-2-phthalimido-2-deoxy-3-*O*-acetyl-6-benzyl-1-thiophenyl- $\beta$ -D-glucopyranoside, **184**, (0.6 g, 0.6 mmol) in anhydrous DCM (10.0 mL) was cooled to 0°C and treated with 4Å molecular sieves, trimethylsilyl azide (0.2 mL, 1.5 mmol), NIS (91.0 mg, 1.5 mmol) and *p*-toluenesulfonic acid (11.0  $\mu$ L, 0.2 mmol).



The reaction was gradually allowed to warm to room temperature and then stirred under an atmosphere of N<sub>2</sub> for 16h. The dark red solution was diluted with a saturated solution of sodium thiosulphate (20.0 mL). The organic layer was separated, washed with water (20.0 mL), a saturated solution of NaHCO<sub>3</sub> (20.0 mL) and brine (20.0 mL). The organic layer was dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield a yellow syrup. The crude mixture was separated by column chromatography on silica using petroleum ether:ethyl acetate, 6:4, as the eluent but no product was isolated.

## 5. Bibliography.

- (1) M. Fukuda, *Mol. Glycobiol.*, 1994, 1-52.
- (2) P. Wothers, N. Greeves, S. Warren and J. Clayden, '*Organic Chemistry*', Oxford University Press, 2001, 1-1512.
- (3) A. Kobata, *Acc. Chem. Res.*, 1993, **26**, 319-24.
- (4) R. A. Dwek, *Chem. Rev.*, 1996, **96**, 683-720.
- (5) R. Kornfeld and S. Kornfeld, *Ann. Rev. Biochem.*, 1985, **54**, 631-64.
- (6) B. Imperiali and S. E. O. Connor, *Curr. Opin. Chem. Biol.*, 1999, **3**, 643-49.
- (7) N. Sharon and H. Lis, *Science*, 1989, **246**, 227-34.
- (8) H. Lis and N. Sharon, *Chem. Rev.*, 1998, **98**, 637-74.
- (9) I. Ofek and N. Sharon, *Curr. Top. Microbiol. Immunol.*, 1990, **151**, 91-113.
- (10) A. Varki and L. D. Powell, *J. Biol. Chem.*, 1995, **270**, 14243-46.
- (11) P.R. Crocker, S. Kelm, A. Hartnell, S. Freeman, D. Nath, M. Vinson and S. Mucklow, *Biochem. Soc. Trans.*, 1996, **24**, 150-56.
- (12) W.I. Weiss, K. Drickamer and W. A. Hendrickson, *Nature*, 1992, **360**, 127-34.
- (13) L. A. Lasky, *Annu. Rev. Biochem.*, 1995, **64**, 113-39.
- (14) D. Zopf and S. Roth, *Lancet*, 1996, **347**, 1017-21.
- (15) G.V. Childs, C. Hyde, Z. Naor and K. Catt, *Endocrinology*, 1983, **113**, 2120-28.
- (16) E.G. Rennels and D. C. Herbert, *Int. Rev. Physiol.*, 1980, **22**, 1-40.
- (17) J.G. Pierce and T. F. Parsons, *Ann. Rev. Biochem.*, 1981, **50**, 465-95.
- (18) N.K. Kalyan and O. P. Bahl, *J. Biol. Chem.*, 1983, **258**, 67-74.



- (19) F.O. Calvo and R. J. Ryan, *Biochemistry*, 1985, **24**, 1953-59.
- (20) T.W. Rademacher, R.B. Parekh and R. A. Dwek, *Annu. Rev. Biochem.*, 1988, **57**, 785-838.
- (21) R.G. Spiro, Q. Zhu, V. Bhoyroo and H.-D. Söling, *J. Biol. Chem.*, 1996, **271**, 11588-94.
- (22) B. di Jeso, L. Ulianich, F. Pacifico, A. Leonardi, P. Vito, E. Consiglio, S. Formisano and P. Arvan, *Biochem. J.*, 2003, **370**, 449-58.
- (23) A.R. Patil, C.J. Thomas and A. Surolia, *J. Biol. Chem.*, 2000, **275**, 24348-56.
- (24) M.R. Jackson, M.F. Cohen-Doyle, P.A. Peterson and D. B. Williams, *Science*, 1994, **263**, 384-87.
- (25) U.G. Danilczyk, M.F. Cohen-Doyle and D. B. Williams, *J. Biol. Chem.*, 2000, **275**, 13089-97.
- (26) M. Kapoor, H. Srinivas, E. Kandiah, E. Gemma, L. Ellgaard, S. Oscarson, A. Helenus and A. Surolia, *J. Biol. Chem.*, 2003, **278**, 6194-200.
- (27) A. J. Parodi, *Biochim. et Biophys. Acta*, 1999, **1426**, 287-95.
- (28) B. Tsai, Y. Ye and T. A. Rapoport, *Nat. Rev. Mol. Cell Biol.*, 2002, **3**, 246-55.
- (29) P. Burda and M. Aebl, *Biochim. et Biophys. Acta*, 1999, **1426**, 239-57.
- (30) T.J. Ekström, T. Chojnacki and G. Dallner, *J. Biol. Chem.*, 1987, **262**, 4090-97.
- (31) D.C. Crick, J.S. Rush and C. J. Waechter, *J. Neurochem.*, 1991, **57**, 1354-62.
- (32) A. D. Elbein, *Ann. Rev. Biochem.*, 1987, **56**, 497-534.
- (33) J.R. Couto, T.C. Huffaker and P. W. Robbins, *J. Biol. Chem.*, 1984, **259**, 378-82.

- (34) S.W. Homans, R.A. Dwek and T. W. Rademacher, *Biochemistry*, 1987, **26**, 6571-78.
- (35) S.L. Flitsch, H.L. Pinches, J.P. Taylor and N. J. Turner, *J. Chem. Soc., Perkin Trans. I*, 1992, 2087-93.
- (36) T.C. Huffaker and P. W. Robbins, *Biochemistry*, 1983, **80**, 7466-70.
- (37) J.F. Cipollo, R.B. Trimble, J.H. Chi, Q. Yan and N. Dean, *J. Biol. Chem.*, 2001, **276**, 21828-40.
- (38) J. P. Taylor, 'Synthesis of novel acceptor substrates for mannosyl transferases', PhD Thesis, University of Exeter, 1992.
- (39) R.E. Dempksi and B. Imperiali, *Curr. Opin. Chem. Biol.*, 2002, **6**, 844-50.
- (40) P. Burda, S. te Heesen, A. Brachat, A. Wach, A. Dusterhoft and M. Aebi, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 7160-65.
- (41) M. Aebi, J. Gassenhuber, S. te Heesen and H. Domdey, *Glycobiology*, 1996, **6**, 439-44.
- (42) M.J. Spiro, R.G. Spiro and V. D. Bhoyroo, *J. Biol. Chem.*, 1979, **254**, 7668-74.
- (43) L. Lehle and E. Bause, *Biochim. et Biophys. Acta*, 1984, **799**, 246-51.
- (44) K.W. Runge, T.C. Huffaker and P. W. Robbins, *J. Biol. Chem.*, 1984, **259**, 412-17.
- (45) A.J. Parodi and L. A. Quesada-Allue, *J. Biol. Chem.*, 1982, **257**, 7637-40.
- (46) A. Katial, C. Prakash and I. Vijay, *Eur. J. Biochem.*, 1984, **141**, 521-26.
- (47) A.J. Parodi and J. Martin-Barrientos, *Biochem. Biophys. Res. Commun.*, 1984, **118**, 1-7.
- (48) B. Hoflack, R. Cacan and A. Verbert, *Eur. J. Biochem.*, 1981, **117**, 285-90.



- (49) L. Revers, '*Studies on the expression, purification and synthetic utility of recombinant yeast 1,4-mannosyltransferase*', PhD Thesis, Merton College, 1996.
- (50) B. Imperiali and T. L. Hendrickson, *Bioorg. Med. Chem.*, 1995, **3**, 1565-78.
- (51) E. Bause, *Biochem. J.*, 1983, **209**, 331-36.
- (52) I. Mononen and E. Karjalainen, *Biochim. et Biophys. Acta*, 1984, **788**, 364-67.
- (53) G. von Heijne and I. Nilsson, *J. Biol. Chem.*, 1993, **268**, 5798-801.
- (54) G. Bause and G. Legler, *Biochem. J.*, 1981, **195**, 639-44.
- (55) D.D. Pless and W. J. Lennarz, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 134-38.
- (56) D.D. Carson, B.J. Earles and W. J. Lennarz, *J. Biol. Chem.*, 1981, **256**, 11552-57.
- (57) H. Hettkamp, G. Legler and E. Bause, *Eur. J. Biochem.*, 1984, **142**, 85-90.
- (58) D.M. Burns and O. Touster, *J. Biol. Chem.*, 1982, **257**, 9991-10000.
- (59) M.L. Reitman, I.S. Trowbridge and S. Kornfeld, *J. Biol. Chem.*, 1982, **257**, 10357-63.
- (60) M. A. Lehrman, *Glycobiology*, 1991, **1**, 553-62.
- (61) S. Silberstein and R. Gilmore, *FASEB J.*, 1996, **10**, 849-58.
- (62) H. Paulsen, *Angew. Chem. Int. Ed. Engl.*, 1990, **29**, 823-938.
- (63) R. R. Schmidt, *Angew. Chem. Int. Ed. Engl.*, 1986, **25**, 212-35.
- (64) H. Paulsen, *Angew. Chem. Int. Ed. Engl.*, 1982, **21**, 155-73.
- (65) S.L. Flitsch and G. M. Watt, '*Chemical synthesis of glycoprotein glycans*', Marcel Dekker Inc., 1997, 207-44.

- (66) J.J. Gridley and H. M. I. Osborn, *J. Chem. Soc., Perkin Trans. I*, 2000, 1471-91.
- (67) M.A.E. Shaban and R. W. Jeanloz, *Carbohydr. Res.*, 1976, **52**, 103-14.
- (68) S. David, A. Malleron and C. Dini, *Carbohydr. Res.*, 1989, **188**, 193-200.
- (69) H. Kunz and W. Günther, *Angew. Chem. Int. Ed. Engl.*, 1988, **27**, 1086-87.
- (70) N. Yamazaki, E. Eichenberger and D. P. Curran, *Tetrahedron Lett.*, 1994, **35**, 6623-26.
- (71) N.K. Kochetkov, V.I. Torgov, N.N. Malysheva and A. S. Shashkov, *Tetrahedron*, 1980, **36**, 1099-105.
- (72) F. Barresi and O. Hindsgaul, *J. Am. Chem. Soc.*, 1991, **113**, 9376-77.
- (73) D. Crich and S. Sun, *Tetrahedron*, 1998, **54**, 8321-48.
- (74) O.J. Plante, R.B. Andrade and P. H. Seeberger, *Org. Lett.*, 1999, **1**, 211-14.
- (75) O.J. Plante, E.R. Palmacci and P. H. Seeberger, *Org. Lett.*, 2000, **2**, 3841-43.
- (76) Y. Ichikawa, G.C. Look and C.-H. Wong, *Anal. Biochem.*, 1992, **202**, 215-38.
- (77) L. F. Leloir, *Science*, 1971, **172**, 1299-303.
- (78) E.J. Toone, E.S. Simon, M.D. Bednarski and G. M. Whitesides, *Tetrahedron*, 1989, **45**, 5365-422.
- (79) S. Sabesan and J. C. Paulson, *J. Am. Chem. Soc.*, 1986, **108**, 2068-80.
- (80) M.M. Palcic, O.P. Srivastava and O. Hindsgaul, *Carbohydr. Res.*, 1987, **159**, 315-24.
- (81) U. Zehavi, S. Sadeh and M. Herchman, *Carbohydr. Res.*, 1983, **124**, 23-34.
- (82) G.M. Watt, L. Revers, M.C. Webberly, I.B.H. Wilson and S. L. Flitsch, *Carbohydr. Res.*, 1998, **305**, 533-41.



- (83) S. Singh, M. Scigelova and D. H. G. Crout, *J. Chem. Soc., Chem. Commun.*, 1996, 993-94.
- (84) N. Taubken and J. Thiem, *Synthesis*, 1991, 517-19.
- (85) L. Revers, I.B.H. Wilson, M.C. Webberly and S. L. Flitsch, *Biochem. J.*, 1994, **299**, 23-27.
- (86) L. Jaenicke and H.-U. Siegmund, *Biol. Chem. Hoppe-Seyler*, 1986, **367**, 787-95.
- (87) S.L. Flitsch, D.M. Goodridge, B. Guilbert, L. Revers, M.C. Webberly and I. B. H. Wilson, *Bioorg. Med. Chem.*, 1994, **2**, 1243-50.
- (88) P. Orlean, *Biochem. Cell Biol.*, 1992, **70**, 438-47.
- (89) C.F. Albright, P. Orlean and P. W. Robbins, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 7366-69.
- (90) T. Mankowski, W. Jankowski, T. Chojnacki and P. Franke, *Biochemistry*, 1976, **15**, 2125-30.
- (91) S. Suzuki, F. Mori, T. Takigawa, K. Ibata, Y. Ninagawa, T. Nishida, M. Mizuno and Y. Tanaka, *Tetrahedron Lett.*, 1983, **24**, 5103-06.
- (92) B. Imperiali and J. W. Zimmerman, *Tetrahedron Lett.*, 1988, **29**, 5343-44.
- (93) G. Palamarczyk, L. Lehle, T. Mankowski, T. Chojnacki and W. Tanner, *Eur. J. Biochem.*, 1980, **105**, 517-23.
- (94) P. W. Atkins, '*Physical Chemistry*', Oxford University Press, 1997, 1-1031.
- (95) A.F. Clark and C. L. Villemez, *FEBS Lett.*, 1973, **32**, 84.
- (96) I. Sprung, L. Carmes, G.M. Watt and S. L. Flitsch, *ChemBioChem*, 2003, **4**, 319-32.

- (97) J. Xing, W.T. Forsee, E. Lamani, S.D. Maltsev, L.L. Danilov, V.N. Shibaev, J.S. Schutzbach, H.C. Cheung and M. J. Jedrzejewski, *Biochemistry*, 2000, **39**, 7886-94.
- (98) I. Sprung, A. Ziegler and S. L. Flitsch, *J. Chem. Soc., Chem. Commun.*, 2002, 2676-77.
- (99) M.A. Warpehoski, B. Chabaud and K. B. Sharpless, *J. Org. Chem.*, 1982, **47**, 2897-900.
- (100) M.A. Umbreit and K. B. Sharpless, *J. Am. Chem. Soc.*, 1977, **99**, 5527-28.
- (101) W. Oppolzer, R.N. Radinov and E. El-Sayed, *J. Org. Chem.*, 2001, **66**, 4766-70.
- (102) J.W. Perich and R. B. Johns, *Synthesis*, 1987, 142-44.
- (103) M.M. Sim, H. Kondo and C.-H. Wong, *J. Am. Chem. Soc.*, 1993, **115**, 2260-67.
- (104) P. Duret, R. Hocquemiller, J.-C. Gantier and B. Figadere, *Bioorg. Med. Chem.*, 1999, **7**, 1821-26.
- (105) S. Takano, T. Sato, K. Inomata and K. Ogasawara, *J. Chem. Soc., Chem. Commun.*, 1991, **7**, 462-64.
- (106) J. Mulzer and C. Brand, *Tetrahedron*, 1986, **42**, 5961-68.
- (107) S.G. Alvarez and M. T. Alvarez, *Synthesis*, 1997, 413-14.
- (108) S.G. Alvarez, G.B. Fisher and B. Singavam, *Tetrahedron Lett.*, 1995, **36**, 2567.
- (109) S. Nagarajan and B. Ganem, *J. Org. Chem.*, 1987, **52**, 5044-46.
- (110) H. Bayley, D.N. Standring and J. R. Knowles, *Tetrahedron Lett.*, 1978, **39**, 3633-34.



- (111) A. Koziara, K. Osowska-Pacewicka, S. Zawadzki and A. Zwierzak, *Synthesis*, 1985, 202-04.
- (112) A. Kamal, P.S.M.M. Reddy and D. R. Reddy, *Tetrahedron Lett.*, 2002, **43**, 6629-31.
- (113) A. Kamal, K.V. Ramana, H.B. Ankati and A. V. Ramana, *Tetrahedron Lett.*, 2002, **43**, 6861-63.
- (114) Y.G. Gololobov, I.N. Zhmurova and L. F. Kasukhin, *Tetrahedron*, 1981, **37**, 437-72.
- (115) R. P. Haughland, 'The Handbook of Chemical Probes', 2002, 253.
- (116) R. Baker and P. M. Winton, *Tetrahedron Lett.*, 1980, **21**, 1175-78.
- (117) S.G. Van Ornum and J. M. Cook, *Tetrahedron Lett.*, 1996, **37**, 7185-88.
- (118) K.R. Edvardsen, T. Benneche and M. A. Tius, *J. Org. Chem.*, 2000, **65**, 3085-89.
- (119) S.R. Gilbertson, G.S. Hoge and D. G. Genov, *J. Org. Chem.*, 1998, **63**, 10077-80.
- (120) L.F. Tietze, U. Beifuss and M. Ruther, *J. Org. Chem.*, 1989, **54**, 3120-29.
- (121) W. Fraser, C.J. Suckling and H. C. S. Wood, *J. Chem. Soc. Perkin Trans. I*, 1990, 3137-44.
- (122) M.C. Pirrung, H. Han and R. T. Ludwig, *J. Org. Chem.*, 1994, **59**, 2430-36.
- (123) K. Ito, R. Kashiwagi, S. Hayashi, T. Uchida and T. Katsuki, *Synlett*, 2001, **2**, 284-86.
- (124) L. Crombie, W.M.L. Crombie and P. Tuchinda, *J. Chem. Soc. Perkin Trans. I*, 1988, 1255-62.

- (125) K.C. Nicolaou and H. J. Mitchell, *Angew. Chem. Int. Ed. Engl.*, 2001, **40**, 1576-624.
- (126) F. Schmitt and P. Sinay, *Carbohydr. Res.*, 1973, **29**, 99-111.
- (127) C.D. Warren and R. W. Jeanloz, *Carbohydr. Res.*, 1977, **53**, 67-84.
- (128) C. Bosso, J. Defaye, A. Domard and A. Gadelle, *Carbohydr. Res.*, 1986, **156**, 57-68.
- (129) S.-I. Nishimura and H. Kuzuhara, *Carbohydr. Res.*, 1989, **194**, 223-31.
- (130) R.U. Lemieux and R. M. Radcliffe, *Can. J. Chem.*, 1979, **57**, 1244-51.
- (131) D. Macmillan, 'The synthesis of novel homogeneous glycoproteins', PhD Thesis, University of Edinburgh, 1999.
- (132) W. Kinzy and R. R. Schmidt, *Liebigs. Ann. Chem.*, 1985, 1537-45.
- (133) D. Chaplin, D.H.G. Crout, S. Bornemann, D.W. Hutchinson and R. Khan, *J. Chem. Soc. Perkin Trans. I*, 1992, 235-37.
- (134) H. Dorta, *Helv. Chim. Acta.*, 2000, **83**, 119.
- (135) T.K. Park, I.J. Kim, S. Hu, M.T. Bilodeau, J.T. Randolph, O. Kwon and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1996, **118**, 11488-500.
- (136) T. Nakano, Y. Ito and T. Ogawa, *Carbohydr. Res.*, 1993, **243**, 43-69.
- (137) M.P. DeNinno, J.B. Etienne and K. C. Duplantier, *Tetrahedron Lett.*, 1995, **36**, 669-72.
- (138) I. Matsuo, M. Isomura, R. Walton and K. Ajisaka, *Tetrahedron Lett.*, 1996, **37**, 8795-98.
- (139) K.C. Nicolaou, R.E. Dolle, D.P. Papahatjis and J. L. Randall, *J. Am. Chem. Soc.*, 1984, **106**, 4189-92.



- (140) T. Matsumoto, H. Maeta, K. Suzuki and G. Tsuchihashi, *Tetrahedron Lett.*, 1988, **29**, 3567-70.
- (141) K.C. Nicolaou, N.J. Bockovich, D.R. Carcanague, R.W. Hummel and L. F. Even, *J. Am. Chem. Soc.*, 1992, **114**, 8701-02.
- (142) E. Meinjohanns, M. Meldal, H. Paulsen and K. Bock, *J. Chem. Soc., Perkin Trans. I*, 1995, 405-15.
- (143) B. Paul, R.J. Bernacki and W. Korytnyk, *Carbohydr. Res.*, 1980, **80**, 99-115.
- (144) E. Walker-Nasir and R. W. Jeanloz, *Carbohydr. Res.*, 1979, **68**, 343-63.
- (145) M. Kiso and L. Anderson, *Carbohydr. Res.*, 1985, **136**, 309-23.
- (146) R.K. Jain and K. L. Matta, *Carbohydr. Res.*, 1992, **226**, 91-100.
- (147) T. Ogawa, S. Nakabayashi and k. Sasajima, *Carbohydr. Res.*, 1981, **95**, 308-12.